EDITORIAL

Milton Jay Greenman

On April 7, 1937, passed away this rare personality and capable administrator of The Wistar Institute of Anatomy and Biology. Doctor Greenman gave evidence early in his career of being a man of unusually liberal ideas. An institute which in the beginning was a museum has evolved under his guidance into a large establishment for breeding of standardized animals, a valuable publishing enterprise, and a research institution with a biological farm as an adjunct, which is the first of its kind in the world. Only a mind receptive to new ideas and an order of administrative ability quite out of the ordinary could have blended and integrated these various interests of the Institute into a successfully working unit. It is not too much to say that The Wistar Institute can now provide standardized research animals of many kinds for biological investigation, can supply laboratory equipment and housing in which to carry on the investigations and finally publish the results to the world in any one of eight journals 'all under one roof' so to speak. This singular combination of resources is unique in research institutions.

Readers of The Journal of Nutrition probably are not as familiar as they should be either with the present character of The Wistar Institute or with the man who has been chiefly responsible for its metamorphosis. Doubtless more adequate memorial publications regarding Doctor Greenman will appear in due time. The following data have been gathered from material supplied by Miss G. L. Lawton of the Institute Staff.

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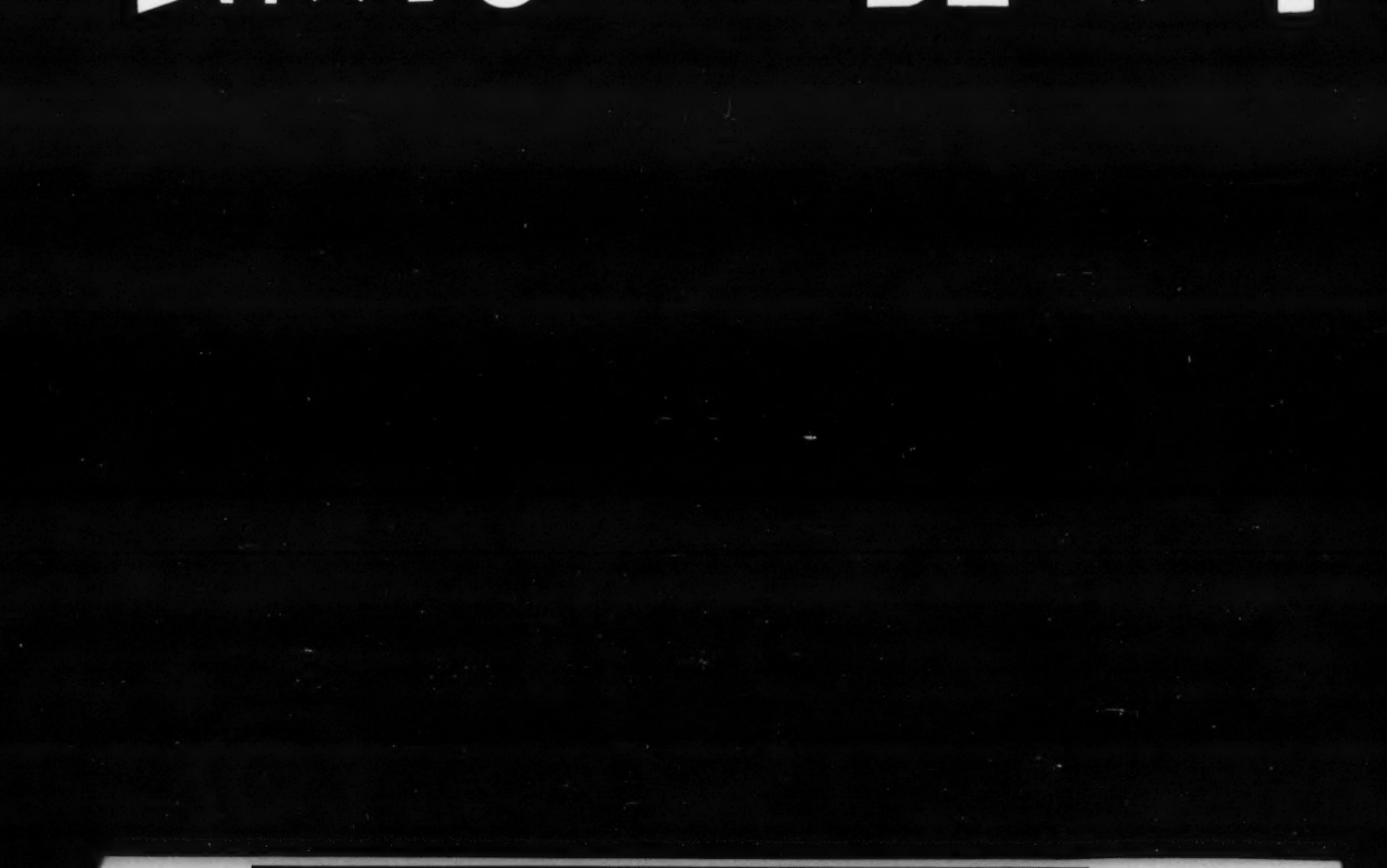
W. J. Greenman



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degree in 1889, and from the School of Medicine with the M.D. degree in 1892. University of Pittsburgh conferred on him its honorary Sc.D. in 1912 and the University of Pennsylvania the same degree in 1927.

He was assistant instructor in general biology, University of Pennsylvania, 1887 to 1889; instructor in biology, 1889 to 1892 while pursuing his medical studies; and lecturer on physiology, Biological School, University of Pennsylvania from 1892 to 1893.

He became Assistant Director of The Wistar Institute in 1892 under Dr. Harrison Allen, the first director. Upon Doctor Allen's resignation in July, 1894, Doctor Greenman continued as Assistant Director with Dr. Horace Jayne until the resignation of Doctor Jayne in December, 1904. Doctor Greenman was elected Director of The Wistar Institute in January, 1905.

The respect in which Doctor Greenman was held as an experienced administrator is indicated by the demand upon his services as trustee of the following institutions: Marine Biological Laboratory, Woods Hole, Massachusetts; Laboratory of the Biological Survey of the Mount Desert region, Bar Harbor, Maine; Vineland Training School, Vineland, New Jersey; Yale Laboratories of Primate Biology, Orange Park, Florida; also as a member of the Council and Publication Committee of the Philadelphia Academy of Natural Sciences.

Membership in the following societies indicate the breadth of his interest as well as his competency in science: American Society of Naturalists, American Association of Anatomists, Eugenics Research Association, Academy of Natural Sciences of Philadelphia, American Association for the Advancement of Science (Fellow), the College of Physicians of Philadelphia (Fellow). Honorary societies which conferred membership on him were Sigma Xi, and the American Philosophical Society. He was a corresponding member of the Peking Society of Natural History, an Honorary member of the Sociedad Cubana de Historia Natural, 'Felipe Poey,' 'First Patron' of the Anatomical and Anthropological Association of China.

Doctor Greenman was a competent investigator, although his preoccupation with administrative duties prevented full fruition of his talents in the research field. Articles on the regeneration of peripheral nerves appeared from his pen in The Journal of Comparative Neurology ('13, '17) and in the Journal of Nervous and Mental Diseases ('16). Several technological articles appeared in The Anatomical Record ('07, '08, '15). The remainder of his publications concerned his beloved Wistar Institute (Bulletin nos. 1, 3, 5, 6, 7, 8 and articles in the Proceedings of the American Association of Museums 1912, 1913; the Rockefeller Series on methods and problems of Medical Research 1930; the University of Pennsylvania General Magazine and Historical Chronicle, 1931).

Doctor Greenman was always first to disclaim credit for all the advances made by The Wistar Institute under his directorship. A few days after his succession in 1905 he called a conference of representative anatomists of the country for the purpose of outlining "a policy whereby the Institute might participate to a greater degree in the promotion of American biological research." This resulted in the establishment of an Advisory Board as a permanent feature of the Institute's organization. The original ten were the following, G. Carl Huber, Lewellys F. Barker, Henry H. Donaldson, Edwin G. Conklin, Franklin P. Mall, J. Playfair McMurrich, George S. Huntington, Charles S. Minot, Simon H. Gage and George A. Piersol. This Board (naturally changing as to personnel) has met regularly once a year, usually at the Institute, to discuss its scientific policy and make recommendations to the Board of Managers. In Doctor Greenman's own words the Advisory Board has suggested continuously "the type and field of research which the Institute could most effectively follow." [1] He credits the Board, and in particular Prof. E. G. Conklin, with the idea of having The Wistar Institute give support to scientific publications. Through the Board's "efforts the several publications issued by the Institute have

[1] Bulletin no. 6 of The Wistar Institute, Commemorating the Twentieth Anniversary of the Organization of the Advisory Board, 1925, p. 36.

been brought together under one management," and the Institute has been brought into closer cooperation with various societies supporting the journals. Doctor Greenman commends "the peculiar wisdom of extending the field of research of an existing institution rather than establishing a new one" [2] adopted by the Board of Managers; also the wisdom of establishing its own press equipment, whereby a deficit in 1922 of nearly $12,000 in the cost of publishing its six journals, was converted into a profit in 1924 of $582. To readers of this Journal the last comment will be of particular interest because it explains how a research institution can print and publish under its own roof superior journals at less cost to its subscribers than can a commercial press. Economies are effected not only by adopting a uniform format for several journals and by preparing copy in a uniform manner, but also in the purchase of stock and in the amount of clerical work required per journal.

While Doctor Greenman very generously credits these policies to the two Boards with which he was constantly associated, the members of the Boards in turn at every opportunity gave full credit to Doctor Greenman. For example, Mr. Effingham B. Morris, recent President of the Board of Managers in his introductory remarks at the twentieth anniversary celebration referred to above, said, "I would like to say a word (before introducing him) about Doctor Greenman. You know he is the most modest man that you can find in a day's walk. In addition to all this work that you know about . . . . there are many other things that Doctor Greenman has been directing in his own quiet way." [3] Then he went on to speak of a line of investigation in which one would not expect the Wistar to be engaged.

Likewise Dr. H. H. Donaldson in his report on the research activities of the Wistar at the 1925 celebration said, "if we have succeeded, in a measure, in carrying out our research program, it has been largely accomplished through the constant cooperation of the Director of the Institute—Doctor

[2] Ibid p. 38.

[3] Supplement to Bulletin no. 6, p. 9.

Greenman—a natural investigator temporarily engaged in administering a scientific institute."[4]

Dr. C. E. McClung in speaking of the "Changes in Method of Biological Research as pursued by Museums and Possibilities of the Future," said "It is possible to adopt the autocratic method and put in charge a man of strong personality and intense convictions and authorize him to carry forward a personal program. This gets results of a definite kind and is an efficient method, but it depends upon one man and is limited by his ability and productivity. There is the other method of reaching out and making numerous contacts and thereby profiting from the abilities of many people, while at the same time a corresponding return is made to them. This is not so easy as the one-man method, because the involvement of numbers means the increase of mutual adjustments and that necessarily complicates matters. But it can be done, and The Wistar Institute has shown the way by its last twenty years of operation. As a result it has had an influence all out of proportion to its size, because, through the foresight of its Director, it elected to render the service that many desired, rather than the one which he determined."[5]

Readers of the Journal may not be aware that Doctor Greenman had been for many years intensely interested in the subject of nutrition, and it was this interest that made him immediately receptive to the idea first expressed to the writer by Dr. Lafayette B. Mendel, member of our Editorial Board, that The Wistar Institute purchase the Journal. The details of this transaction have been related in volume 7, page 365 of this journal. It was also through his efforts that a Department of Nutrition has recently been established in a small way in The Wistar Institute. All of our readers will be interested in the further progress of this department.

The chief pride of the Director in recent years has been the development of The Effingham B. Morris Biological Farm near Bristol, Pa. A visit will well repay students of nutrition, for there is already in operation a rat colony building, an

[4] Bulletin no. 6, p. 51.
[5] Ibid p. 72.

opossum colony and colonies of various amphibian forms—all of which would lend themselves admirably to investigation of many problems in nutrition. The idea of this farm originated from the necessity of supplying standard feeds for the rat colony in Philadelphia. It has however grown far beyond the original conception, due quite obviously to the open-minded enthusiasm of Doctor Greenman for new and progressive ideas.

How The Wistar Institute Managers will meet the emergency created by Doctor Greenman's death remains to be seen. It is the personal opinion of the writer that any change in personality of the Directorship will not materially alter the pleasant associations already existing between the Editorial Board and The Wistar Institute. The publication policy has been so successful that it is sure to be continued substantially along the lines which now exist.

Doctor Greenman, however, will be sorely missed. His charming manner and infectious enthusiasm for the various projects of the Institute were of great influence in securing harmonious cooperation. One of his admirers writes, "No one can take his place whether in ability or in sympathy and help extended to young workers. I owe much to him and his encouragement. The only way is to pass it on to other young workers."

JOHN R. MURLIN

HEMOGLOBIN REGENERATION IN ANEMIC RATS IN RELATION TO IRON INTAKE

WITH SUGGESTIONS FOR IMPROVEMENT OF THE BIOASSAY TECHNIC FOR MEASURING AVAILABLE IRON

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ONE FIGURE

(Received for publication December 7, 1936

Interest in the determination of the availability for hemoglobin formation of the iron in foodstuffs has resulted from the finding that all compounds of iron are not equally effective in the remission or prevention of nutritional anemia (Elvehjem et al., '33). The bioassay method of measuring the extent to which the iron in a food is utilizable for hemoglobin consists in comparing the gains in hemoglobin in anemic rats fed a food source of iron, with the hemoglobin regeneration in litter mate anemic rats given the same amount of iron as iron chloride, both food and iron chloride being amply supplemented with copper. In the method as developed by Elvehjem et al. ('33), the comparison is made at a 0.3 mg. daily level of iron feeding, an amount of available iron which has been found to raise the hemoglobin to the normal level in a 6-week test period.

In the course of our bioassay measurements of the hematopoietic value of food stuffs, we encountered the difficulty of measuring the availability of iron in foods of low iron content due to the inability of the rat to consume a large enough portion to obtain the required 0.3 mg. of iron daily.

As pointed out in a previous paper (Smith and Otis, '36) it does not seem necessary to compare the availability of iron in a food with Fe as $FeCl_3$ at this particular level. It is logical to believe that as long as the iron content of the food portion fed is not higher than is necessary to promote complete regeneration in the test period, it should be possible to make comparisons of availability at any iron level which is present in a readily consumable portion of the food to be tested—that is—at iron levels below that which is necessary for complete regeneration of hemoglobin. Accordingly, it seemed advisable to measure the hemoglobin regeneration in young anemic rats at different levels of iron intake, and the results of this study are reported in this paper.

EXPERIMENTAL PROCEDURE

Preparation of the test animals. Following the procedure developed by Elvehjem and Kemmerer ('31), young rats which had access only to whole milk, were weaned at 3 weeks of age and continued upon the milk ration until a definite degree of anemia developed. The degree of anemia was determined by hemoglobin measurements (using the Newcomer method) of blood obtained from the tail. Young rats in our laboratory handled according to the Elvehjem-Kemmerer technic, except that the litters were not reduced in size, reached an average hemoglobin level of 3.9 gm. per 100 cc., usually between the fourth and fifth week after weaning. The supplemental iron or food feeding was then begun and the regeneration in hemoglobin measured bi-weekly during the subsequent 6-week test period. In every case 0.05 mg. of copper as $CuSO_4$ and 0.04 mg. of manganese as $MnCl_2$ were given in addition to the iron or the food supplement. The iron was fed as a solution of iron chloride whose iron content had been determined by the thiocyanate method of analysis (Kennedy, '27). It was measured directly from a micro-burette onto a small portion of powdered milk which the rats consumed greedily. The iron free manganese chloride and copper sulfate supplements were given at the same time. The animals were housed

individually in heavily galvanized metal mesh cages with raised screen bottoms. Whole milk and distilled water provided in glass containers were given ad libitum.

The gains in hemoglobin at levels of iron intake varying from 0.014 mg. to 0.3 mg. daily, appear in table 1, and are shown graphically in figure 1.

TABLE 1

Hemoglobin gains in anemic rats fed different amounts of iron as iron chloride

AMOUNT OF IRON FED DAILY [1]	NUMBER OF RATS USED IN STUDY	INITIAL HEMOGLOBIN	GAINS IN HEMOGLOBIN (GRAMS PER 100 CC.)		
			In 2 weeks	In 4 weeks	In 6 weeks
			± Probable error	± Probable error	± Probable error
A. Males					
mg.		*gm. per 100 cc.*			
0.014	14	4.0	0.4 ± 0.16	0.7 ± 0.07	1.1 ± 0.18
0.028	13	3.9	1.0 ± 0.08	1.6 ± 0.12	2.2 ± 0.25
0.050	20	4.0	1.3 ± 0.11	2.4 ± 0.13	3.5 ± 0.14
0.071	5	3.9	1.7 ± 0.30	3.3 ± 0.27	4.3 ± 0.24
0.10	14	4.0	2.4 ± 0.16	3.8 ± 0.13	5.5 ± 0.14
0.15	11	4.0	3.5 ± 0.17	5.6 ± 0.11	8.2 ± 0.21
0.20	12	3.8	3.8 ± 0.12	6.7 ± 0.21	9.1 ± 0.19
0.25	12	3.5	5.5 ± 0.17	8.4 ± 0.15	10.4 ± 0.18
0.30	7	4.1	5.1 ± 0.30	8.6 ± 0.27	10.1 ± 0.29
B. Females					
0.014	5	4.1	1.0 ± 0.20	1.8 ± 0.30	2.3 ± 0.32
0.028	14	4.1	1.5 ± 0.13	2.5 ± 0.12	3.1 ± 0.24
0.050	13	4.4	2.1 ± 0.25	3.0 ± 0.29	4.2 ± 0.33
0.071	15	3.9	2.8 ± 0.17	3.9 ± 0.11	5.6 ± 0.25
0.10	12	4.0	2.7 ± 0.09	5.0 ± 0.17	6.5 ± 0.15
0.15	10	3.75	4.4 ± 0.22	6.6 ± 0.27	9.5 ± 0.26
0.20	8	3.7	5.0 ± 0.27	8.4 ± 0.22	10.9 ± 0.25
0.25	6	4.1	5.7 ± 0.30	9.0 ± 0.28	10.7 ± 0.18
0.30	7	4.6	6.3 ± 0.30	9.3 ± 0.17	10.2 ± 0.16

[1] 0.05 mg. Cu as $CuSO_4$ and 0.04 mg. Mn as $MnCl_2$ given daily in addition to the iron supplement.

Inspection of table 1 reveals the following points: As reported previously from this laboratory, a difference between males and females in their response to the supplementing of their whole milk ration with iron may be noted, hemoglobin regeneration being greater in the females than in the

males given the same amount of iron. This difference between the sexes was consistently noted at the end of the 6-week test period except at levels of iron feeding greater than 0.2 mg. daily. That complete hemoglobin regeneration up to the

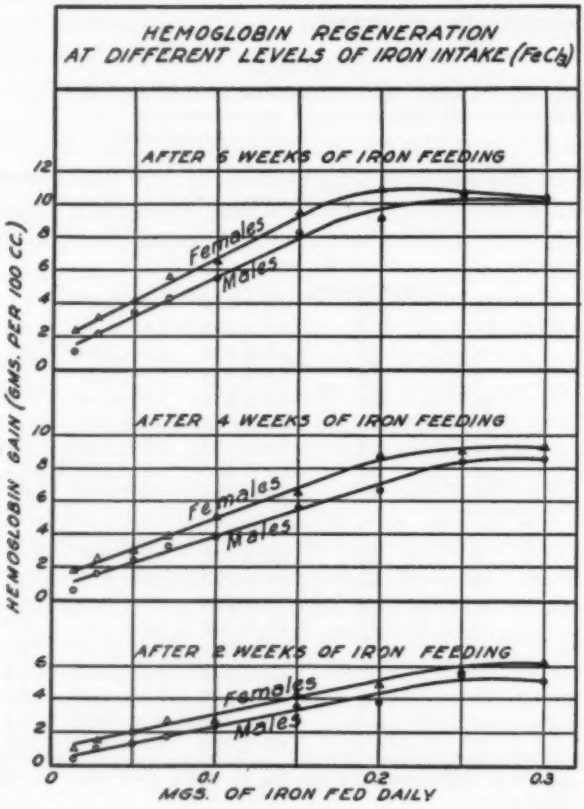

Figure 1

level normal for rats of this age resulted in males from the feeding of 0.25 mg. of iron daily and in females from the feeding of 0.2 mg. daily iron was evident by the finding that no greater gain in hemoglobin resulted from a higher intake

of iron. With daily iron intakes of 0.25 mg. or more, therefore, no observable differences between the sexes in their response could be expected. At the end of 4 weeks, however, sex differences in hemoglobin gains were observed even at the higher levels of feeding for the regeneration had not reached the maximum level in the shorter period of time.

It may be noted that the magnitude of difference in the hemoglobin gains of males and females was about the same at all levels of iron feedings. It is also apparent that it was in the first 2-week period after the beginning of the iron supplementation that the females showed the greatest hemoglobin gains as compared with the males. In the last 2 weeks of the 6-week test period, the differences were very slight. These facts suggest that the difference may be due to a greater store of iron in the female which became available upon the feeding of the copper given with the iron supplement. It may be noted, however, that the initial hemoglobin concentration, i.e., that at the beginning of the test period, is 4.0 for the ninety females studied and 3.9 for the 108 males. Thus a difference in reserve supply of iron between the males and females was not indicated by a significant difference in the hemoglobin concentration of the blood.

With both male and female anemic animals, at levels of iron below that necessary to induce complete regeneration of hemoglobin, the regeneration of hemoglobin was found to be directly proportional to their supplemental iron intake, although not arithmetically so, for the gains in hemoglobin are greater in proportion to the amount of iron fed at the lower levels of iron intake. It required a greater amount of iron to produce the same increment of hemoglobin gain at the higher levels of iron feeding. For example, when the iron level was raised from 0.014 mg. daily to 0.028 mg. daily, an increase of 0.014 mg. of iron, the gain in hemoglobin in the males increased from 1 to 2.2 gm. per 100 cc. of blood. On the other hand, increasing intake from 0.15 to 0.2, an iron increase of 0.05 mg. resulted in no greater increase (from 8.2 to 9.1) in hemoglobin than that resulting from 0.014 mg.

of iron at the lower level of intake. The relationship between iron intake and hemoglobin gain appears to be practically a linear one between the levels of iron feeding of 0.028 to 0.15 mg. per day. It would appear, therefore, that the anemic rats are more sensitive, i.e., make greater hemoglobin gains in proportion to the iron intake, at iron levels less than 0.15 mg. daily, for at iron levels above 0.15 mg. daily the hemoglobin gains in proportion to the amount of iron fed declined.

Obviously, the extent of regeneration of hemoglobin also varied with the length of the feeding period. At no level of iron feeding tested had the hemoglobin regeneration reached its maximum value in less than 6 weeks. At the end of both 2 and 4 weeks of supplementary feeding, the gains in hemoglobin, though smaller than the 6-week gains in general, are directly proportional to the amount of iron given. The results were, however, less regular after only 2 weeks of iron feeding and the increment of hemoglobin gain per unit weight of iron fed was appreciably less. However, the 4-week gains are quite uniform, and the increment of hemoglobin gains per unit weight of iron were not very much smaller than those obtained at 6 weeks.

DISCUSSION OF RESULTS

In view of tabulated results, the authors of this paper make the following suggestions for the improvement of the Elvehjem method for the measurement of the available iron in foodstuffs by bioassay.

That comparisons of the gains in hemoglobin in anemic rats fed a food source of iron with the hemoglobin gains in litter mate anemic rats given the same amount of iron as iron chloride be made at iron levels below 0.2 mg. of iron daily, preferably in the range of 0.03 to 0.15 mg. daily feeding. The exact level selected should depend upon the amount of iron present in a readily consumable portion of the food under test.

It is obvious not only that more uniform results are obtained when the amount of iron fed is below that necessary to promote complete normal regeneration of hemoglobin, but that

smaller differences in iron availability may be detected as the hemoglobin response per unit weight of iron fed is greater when less than 0.3 mg. of iron is fed daily. At levels of iron feeding of 0.2 mg. or above, the use of a shorter test period in which maximum hemoglobin development is not possible would be preferable. At all levels of iron, a 4-week test period gives results which are quite as uniform as those obtained in a 6-week test period. However, as the increment of gains in hemoglobin per unit weight of iron is somewhat smaller at 4 weeks than at 6 weeks, there is a slight sacrifice of sensitivity of the measurement in selection of the 4-week period. The saving of time resulting from a shortened test period is always an argument in its favor.

Again, as females show a greater gain in hemoglobin than males fed the same amount of iron, it is important that males and females should not be used interchangeably, but that comparisons of iron availability should be confined to members of the same sex.

The authors also suggest the possibility of the use of the curves presented herein, which show graphically the hemoglobin gains resulting from the daily feeding of iron in a completely available form at different levels to male and female anemic animals prepared for the test as herein described, as reference curves from which the amount of available iron in a given food supplement can be read from the curve if the hemoglobin gain resulting from the daily feeding of that food is known.

Table 2 presents the results of measuring the availability of the iron in certain foodstuffs using the Elvehjem method modified as suggested above. Measurements of the availability of iron in both corn and peas have been made at two different iron levels (table 2). It may be seen that when the resulting gains in hemoglobin in each case are compared with the hemoglobin gains obtained by animals of the same sex when the iron in the same amount as that present in the food supplement (see reference curve, fig. 1) was fed as $FeCl_3$ the observed percentages of availability of the iron are substantially the same at both levels of iron feeding. It appears,

TABLE 2

Availability of iron in certain foods

FOOD TESTED	GRAMS OF FOOD SUPPLEMENT	MILLIGRAMS OF Fe[1] IN FOOD SUPPLEMENT	5 WEEKS GAIN IN HEMOGLOBIN (GRAMS PER 100 CC.)		MILLIGRAMS OF Fe AS $FeCl_3$ REQUIRED FOR OBSERVED GAIN IN HEMOGLOBIN		PER CENT AVAILABILITY BY BIOASSAY		PER CENT AVAILABILITY BY DIPYRIDYL
			♂	♀	♂	♀	♂	♀	
Dried peas	2.0	0.1154	5.5	6.7	0.10	0.098	87	85	81
Dried peas	1.0	0.0577	...	4.3	....	0.051	...	88	81
Ground whole corn	4.0	0.0960	4.9	...	0.088	....	92	..	..
Ground whole corn	2.0	0.0480	...	4.0	....	0.046	...	96	..
Dried lima beans	1.5	0.1003	4.8	6.1	0.084	0.086	84	86	93
Ground whole wheat	3.0	0.1569	7.7	9.1	0.148	0.145	94	92	92
Ground rolled oats	3.0	0.1056	5.7	6.8	0.105	0.100	99	95	96
Ground whole barley	3.0	0.1230	4.3	5.2	0.073	0.069	59	56	..
Banana powder	4.0	0.0980	5.6	6.6	0.102	0.096	104	98	95–100[2]
Raw potato	4.0	0.0284	...	3.1	....	0.028	...	98	95–100[2]
Ground rye	3.0	0.1090	6.0	7.0	0.111	0.104	101	95	..
Dried apricots	2.0	0.2220	6.9	...	0.130	....	58	..	98[2]
Raw cabbage	4.0	0.0134	...	2.0	....	0.008	...	60	82[2]
Raisins	4.0	0.1378	...	6.5	....	0.094	...	68	94–97[2]
Dates	4.0	0.0204	1.0	...	0.012	....	59	..	82

[1] Iron analyses made on several samples of each food by thiocyanate method.

[2] As determined by Shackelton and McCance. Biochem. J., vol. 30, p. 582 ('36).

therefore, that this method may be used in determining the availability of the iron in foods of low iron content.

Although the percentages of available iron in the food under test as determined by this method agree closely in most foods with the availability as determined by the dipyridyl method introduced by Hill ('30) and modified by Shackelton and McCance ('36), the available iron as determined by bioassay was found to be notably less in certain foods than that found by the chemical method for determination of ionizible iron. These foods mentioned in this table include apricots, raisins, cabbage and dates. The relative low availability of iron in these foods as actually found by feeding experiments may be due to their relatively high roughage content which serves as a mechanical obstruction to the absorption of the iron. Whatever the cause of the lower availability obtained by bioassay tests, which measure the actual utilization of the iron in the food by the animal body, such measurements would logically be the stronger test of physiological availability.

It may again be noted that the hemoglobin gains resulting from the feeding of the different foods is greater in the females than in the males fed the same food and in the same amounts. However, if comparisons are made between those receiving the food and those rats of the same sex receiving the same amount of iron as iron chloride, the percentage availability of the iron in the food may be seen to be practically the same in males and females. However, let it be noted that if care is not taken to make the comparisons of availability of food iron and iron as iron chloride in animals of the same sex the results would be very different. For example, if the hemoglobin gains of the females fed 3 gm. of wheat daily were compared with the response of the males fed the same amount of iron as iron chloride, 115% of the iron in the wheat would appear to be available. On the other hand, if the response of the males fed the food source of iron was compared with the expected hemoglobin gains of the females given iron in its completely available form, a percentage availability of only 72 would be recorded. Thus it is quite important that this sex difference be recognized in making these comparisons.

If this difference in response between males and females can be explained by assuming a larger store of iron (i.e., Josephs' ('32) mobile tissue iron) in the female which cannot be used for hemoglobin formation because of exhaustion of copper reserves, and which therefore becomes available when copper or a food containing it is fed, then at least part of the hemoglobin regeneration resulting from the feeding of a food would be due to its copper, not iron, content. The necessity of exhaustion of body reserves of iron has been previously stressed by Elvehjem and his co-workers. It seems to the authors, therefore, that a more accurate procedure would be to deplete the iron reserves in the presence of sufficient copper so that the hemoglobin response in the test anemic animals would be a measure only of the utilization of iron. The practicability of this procedure and its effect upon the results is being investigated further.

SUMMARY

Hemoglobin regeneration in anemic rats in relation to their intake of iron given as iron chloride has been measured and the results used as the basis for modification of the method for determining available iron in foods by bioassay.

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THE EFFECT OF GALACTOSE ON THE HUMAN RESPIRATORY QUOTIENT AND ALVEOLAR CARBON DIOXIDE [1]

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FIVE FIGURES

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The effect of galactose on the human respiratory quotient has been well established by Deuel ('27), Cathcart and Markowitz ('27), and Carpenter and Lee ('32 a, '32 b and '32 c). In general, there is a marked rise in the respiratory quotient (R.Q.) within one-half to three-quarters of an hour with a sharp fall soon after, and later a gradual return to the pre-ingestion level. As the R.Q. may rise from an average basal level to above unity, the explanation that the rise is due to the increase of combustion of the carbohydrates cannot suffice. Several investigators have found increases in the lactic acid formation after the ingestion of galactose. Wierzuchowski and Laniewski ('31) found an increase in the lactic acid content of the blood and an increased excretion of lactic acid into the urine during continuous intravenous injection into dogs, Ørskov ('32) noted an increase in blood lactic acid after ingestion of the sugar by dogs, and Koike ('34) observed a marked increase in blood lactic acid after intraperitoneal injection of galactose into rabbits.

In view of the possibility of an increase of lactic acid after the ingestion of galactose, a determination of the changes in alveolar CO_2 in parallel with the changes in R.Q. seemed to

[1] A preliminary report of this material was presented before the American Institute of Nutrition, Washington, D. C., on March 25, 1936.

offer a possibility of finding an explanation of the rise in the R.Q., in part, at least. Several series of experiments have been made with a long trained human subject in which the percentage alveolar CO_2 and the respiratory exchange with particular reference to the R.Q. have been determined before and after the ingestion of galactose, galactose and glucose, and lactose. A series was made with 250 cc. of water as a control, a series each with 25 and 50 gm. of galactose, one with 25 gm. of galactose and 25 gm. of glucose combined, and one with 50 gm. of lactose. The sugars were given in 250 cc. of water. The order of periods was three 15-minute periods before ingestion of the dose followed by twelve 15-minute periods consecutively after the dose was taken. The alveolar air samples were taken every 7 to 8 minutes during the periods. The respiratory exchange was measured by the open circuit method of Carpenter and Fox ('31). The alveolar air was collected by a method previously described (Carpenter and Lee, '33 a). The subject was J.C., 52 years of age, 68.5 kg. in weight, and 167 cm. in height. He had served as a subject of a similar investigation of the effect of glucose and fructose on the alveolar CO_2 and the R.Q. (Carpenter and Lee, '33 b). As in the previous investigation, the experiments were made with the subject in a sitting position and therefore they were not made in a strictly basal condition.

RESPIRATORY QUOTIENT AND ALVEOLAR CARBON DIOXIDE

The results of the determinations of the R.Q. and of the alveolar CO_2 for the five series are plotted in figures 1 to 5. The R.Q.'s are plotted at the middle points of the periods and the alveolar CO_2 percentages at the points of time at which they were taken. The quotients and the percentages for each experiment are plotted with the same convention. The solid lines in each figure represent the averages of all the observations of the R.Q. for the respective periods and the averages of the percentages of the alveolar carbon dioxide. Table 1 gives the correlation coefficients between the R.Q.'s and the accompanying average alveolar CO_2 in the respective periods

TABLE 1

Correlation coefficients between respiratory quotient and alveolar carbon dioxide of J.C.

DATE	NUMBER OF PERIODS	RESPIRATORY QUOTIENTS			ALVEOLAR CARBON DIOXIDE			CORRELATION COEFFICIENT	$P.E._r$
		Average	Range	S.D.	Average, per cent	Range, per cent	S.D.		
250 cc. water									
Dec. 12, 1934	15	0.788	0.759–0.806	0.011	6.31	6.23–6.49	0.077	−0.182	±0.168
Dec. 14, 1934	15	0.789	0.757–0.802	0.011	6.05	5.92–6.26	0.089	+0.052	±0.174
Dec. 31, 1934	15	0.810	0.788–0.833	0.013	6.04	5.88–6.20	0.083	−0.304	±0.158
Jan. 9, 1935	15	0.800	0.779–0.838	0.016	5.99	5.81–6.18	0.082	+0.264	±0.162
Jan. 18, 1935	15	0.809	0.793–0.836	0.013	6.14	6.00–6.28	0.068	−0.256	±0.163
Average	15	0.799	0.775–0.823	0.013	6.11	5.97–6.28	0.080	−0.085	±0.165
25 gm. galactose									
Dec. 10, 1934	15	0.809	0.771–0.897	0.038	6.07	5.80–6.18	0.102	−0.465	±0.137
Dec. 28, 1934	15	0.822	0.775–0.936	0.051	5.91	5.77–6.12	0.119	−0.706	±0.087
Jan. 4, 1935	15	0.847	0.794–0.975	0.058	5.83	5.59–6.06	0.146	−0.471	±0.136
Jan. 11, 1935	15	0.869	0.815–1.007	0.059	6.03	5.71–6.22	0.145	−0.730	±0.081
Jan. 14, 1935	15	0.893	0.837–1.014	0.057	6.17	5.97–6.32	0.111	−0.828	±0.055
Average	15	0.848	0.798–0.966	0.053	6.00	5.77–6.18	0.125	−0.640	±0.099
50 gm. galactose									
Jan. 16, 1935	15	0.883	0.804–0.982	0.067	5.91	5.54–6.14	0.150	−0.607	±0.110
Jan. 28, 1935	15	0.873	0.798–0.975	0.066	5.86	5.62–6.13	0.153	−0.605	±0.110
Jan. 30, 1935	15	0.888	0.790–0.996	0.070	5.76	5.41–5.90	0.141	−0.782	±0.068
Feb. 4, 1935	15	0.899	0.818–1.000	0.069	5.98	5.75–6.19	0.132	−0.625	±0.106
Feb. 6, 1935	15	0.914	0.837–1.015	0.067	5.89	5.50–6.20	0.194	−0.709	±0.086
Average	15	0.891	0.809–0.994	0.068	5.88	5.56–6.11	0.154	−0.666	±0.096
25 gm. galactose and 25 gm. glucose									
Mar. 4, 1935	15	0.890	0.843–1.000	0.054	5.76	5.51–5.95	0.121	−0.627	±0.106
Mar. 13, 1935	15	0.870	0.796–0.983	0.062	6.16	5.89–6.34	0.096	−0.115	±0.172
Mar. 18, 1935	15	0.870	0.810–0.960	0.050	5.88	5.66–6.21	0.115	−0.287	±0.160
Apr. 1, 1935	15	0.880	0.801–0.989	0.060	6.14	5.87–6.41	0.174	−0.694	±0.090
May 1, 1935	15	0.878	0.788–0.993	0.056	5.94	5.79–6.07	0.082	−0.396	±0.147
Average	15	0.878	0.808–0.985	0.056	5.98	5.74–6.20	0.118	−0.424	±0.135
50 gm. lactose									
Dec. 7, 1934	15	0.836	0.765–0.922	0.048	6.17	5.98–6.39	0.112	−0.384	±0.148
Dec. 24, 1934	15	0.871	0.802–0.977	0.052	6.33	6.11–6.47	0.099	−0.555	±0.120
Jan. 2, 1935	15	0.889	0.814–0.981	0.053	6.00	5.77–6.13	0.092	−0.102	±0.172
Feb. 13, 1935	15	0.880	0.816–0.979	0.054	6.11	5.92–6.33	0.113	+0.028	±0.174
Feb. 18, 1935	15	0.880	0.808–0.973	0.055	5.92	5.62–6.03	0.116	−0.162	±0.170
Average	15	0.871	0.801–0.966	0.052	6.11	5.88–6.27	0.106	−0.235	±0.157

in each experiment for the five series. The table includes the pre-ingestion periods as well as those after the ingestion of the dose.

Control series. The R.Q.'s and the alveolar CO_2 percentages for the experiments with 250 cc. of water are plotted in figure 1. The average R.Q. after the ingestion of water, shown in solid line, was slightly lower than in the base-line periods, although there was no general trend toward either a

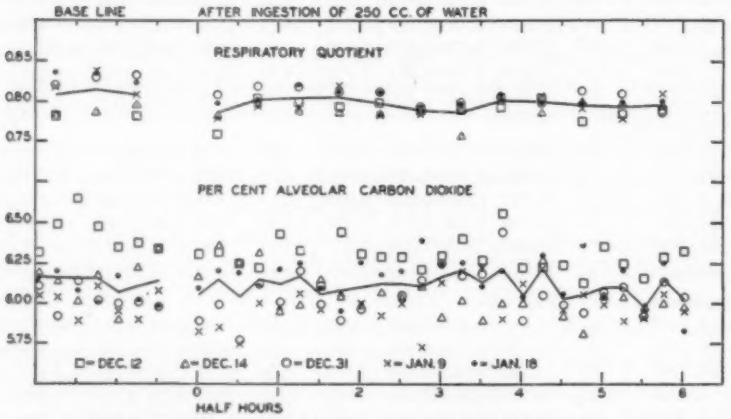

Fig. 1 Respiratory quotients and the percentages of alveolar carbon dioxide before and after the ingestion of 250 cc. of water. The respiratory quotients are plotted at the middle of each period and the carbon dioxide percentages at the points of time at which the samples were taken. The solid lines are the averages of all the experiments.

rise or a fall during the 3 hours. In general, when the base-line quotients were above or below the average line, those after taking water were, for the most part, in the same direction for a particular experiment. This is shown, for example, in the experiments of December 12th and January 9th. The range in R.Q.'s for each experiment is shown in table 1. The standard deviation shows that over two-thirds of the periods differed from the average by less than 0.02. The percentages of alveolar CO_2 show rather wide variations from the average line in figure 1. Part of this is due to the general level of

CO_2 percentages for the day. For example, table 1 shows that the average for December 12th was 6.31%, whereas for January 9th it was 5.99. The maximum range in single determinations was 0.67% on December 31st and the minimum range was 0.50% on January 9th. These ranges are wider than those given in table 1 because in table 1 the ranges are for the averages for the periods whereas figure 1 shows the ranges for the single determinations. There is no valid reason for excluding any single determination shown in figures 1 to 5 and all of the values have been used both for R.Q. and for alveolar CO_2 percentages in making up table 1. The correlation coefficients between the R.Q. and the alveolar CO_2 for the water series are low and without any definite trend toward either the positive or negative side. This finding is not illogical to expect as the measurements were really the slight variations in two physiological functions that were determined when the conditions were such that no marked trends were found. Therefore it is not to be expected that when one of these was slightly below the average the other would also vary in identically the same direction.

Twenty-five grams galactose. The results of the experiments with 25 gm. of galactose are shown in figure 2. Coincident with a marked rise in the R.Q. during the first three quarter-hours was a definite and gradual fall in the alveolar CO_2. This fall continued until just after the R.Q. started to fall. The R.Q. fell to the pre-ingestion level during the fourth half-hour. At the same time there was a rise in the alveolar CO_2. During the third hour the R.Q. tended to fall below the pre-ingestion level and at the same time the alveolar CO_2 was nearly at the pre-ingestion level. In table 1 the correlation coefficients between the alveolar CO_2 and the R.Q. for this series were consistently negative. Three of them were -0.7 and two nearly -0.5. Therefore as the R.Q. rose the alveolar CO_2 fell and vice versa. The greater variations in the R.Q.'s with these experiments than with the control experiments are seen in the ranges and the standard deviations for the R.Q.'s. Similarly the greater variations in the alveolar CO_2 are seen in the standard deviations.

Fifty grams galactose. The results of the five experiments with 50 gm. of galactose are shown in figure 3. A marked rise in the R.Q. was found in the first hour and was accompanied by a marked fall in the alveolar CO_2. There was but a slight fall in the R.Q. during the second hour during which time the alveolar CO_2 rose slightly but fell again to the same low level that was found during the second half-hour. From

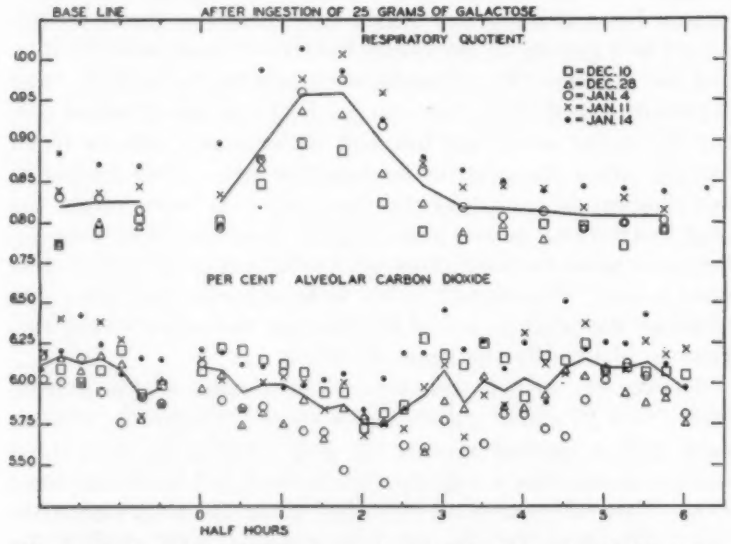

Fig. 2 Respiratory quotients and percentages of alveolar carbon dioxide before and after the ingestion of 25 gm. of galactose in 250 cc. of water. The method of plotting is the same as in figure 1.

the latter part of the third half-hour to the end of the experiment there was a gradual fall in the R.Q. to the pre-ingestion level, which was accompanied by a rise in the alveolar CO_2 to the level at the beginning of the experiment. The correlation coefficients between the R.Q. and the alveolar CO_2 for this series in table 1 are very high and negative. All of them are over -0.6 and two of them are over -0.7. Therefore there was an even more marked relationship between the

changes in the R.Q. and the changes in alveolar CO_2 with 50 gm. of galactose than there was with 25 gm. of galactose.

Twenty-five grams galactose and 25 gm. glucose. The results of the five experiments with 25 gm. of galactose and 25 gm. of glucose (the products of hydrolysis of 50 gm. of lactose) are shown in figure 4. The R.Q. had nearly the same sort of rise as was found with 25 gm. and with 50 gm. of

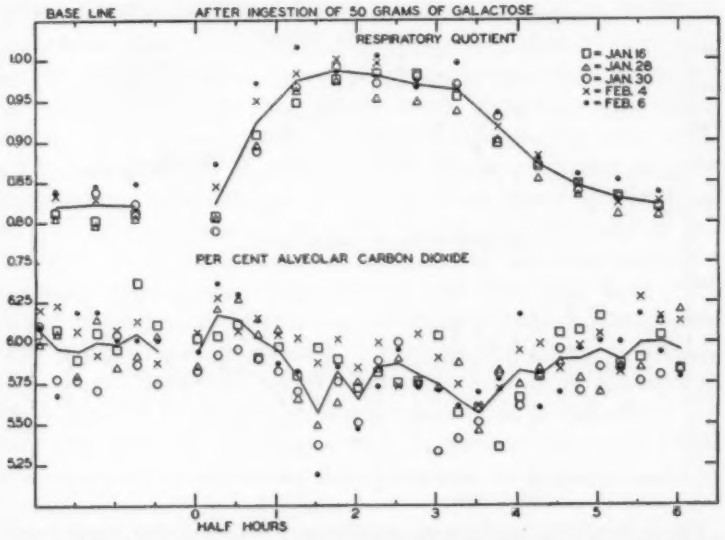

Fig. 3 Respiratory quotients and percentages of alveolar carbon dioxide before and after the ingestion of 50 gm. of galactose in 250 cc. of water. The method of plotting is the same as in figure 1.

galactose. The maximum was reached at the end of the second half-hour, but from there on the fall was more gradual during the next 2 hours than was found for those 2 hours with 25 gm. of galactose, and with 50 gm. of galactose when the fall in R.Q. began. The characteristic curve of the R.Q. after glucose (Carpenter and Fox, '30) is a somewhat more delayed maximum rise and a longer holding up of the curve than with either fructose or galactose. This effect is shown

in this series where the sugars are combined, in that the fall was not so sharp as with galactose alone. There was a tendency for the alveolar CO_2 to fall slightly and there was a generally lower level after the first half-hour until the end of the fifth half-hour. The individual determinations in this series were much more scattered than in any of the preceding series. Part of this is due to the wider range in the alveolar

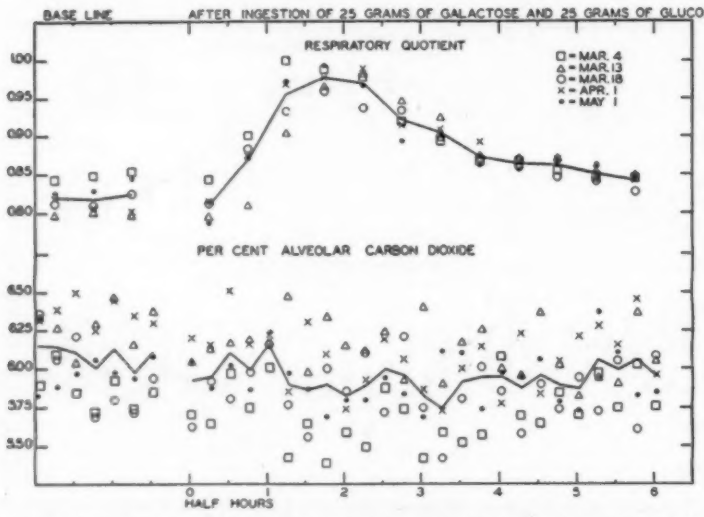

Fig. 4 Respiratory quotients and percentages of alveolar carbon dioxide before and after the ingestion of 25 gm. of galactose and 25 gm. of glucose in 250 cc. of water. The method of plotting is the same as in figure 1.

air level. The averages for this series in table 1 range from 5.76 to 6.16%, but even taking this into account, there is still a great variability in the determinations from one point to another. However, the standard deviations for the alveolar CO_2 in table 1 are not so high as with 25 gm. and 50 gm. each of galactose. Although there is a trend to a lower alveolar CO_2, it is doubtful whether a correlation can be found between the changes in the alveolar CO_2 and the changes in the R.Q., and mathematically this is evident from the variability in the

correlation coefficients in table 1. Two, namely, — 0.627 on March 4th and — 0.694 on April 1st, may be considered significant. Those on March 13th and 18th cannot be considered so and it may be a question as to whether the one on May 1st is significant. Therefore there is a great variability in the response of the alveolar CO_2 to the ingestion of these two sugars given together. The presence of glucose modifies in some way the action of galactose. This finding is in agreement with previous work on the effect of combining glucose with galactose. Folin and Berglund ('22) found that the addition of 100 gm. of glucose reduced the sugar excretion markedly in comparison with that found when galactose was taken alone. Similar results were found by Bodansky with dogs ('32), and Harding and Grant ('33) found a marked drop in galactosuria when 30 gm. of glucose were added to a dose of 40 gm. of galactose. Cori ('26) found that the rate of absorption of galactose from the intestine in rats was markedly reduced when glucose was present and Cori and Cori ('28) found there was a decrease in the galactosuria when glucose was ingested simultaneously. Whether the effect in experiments here reported is due to the slower rate of absorption when glucose is present cannot be stated. It is difficult to see how the presence of glucose and its absorption could affect the action of galactose in the formation of organic acids in its intermediary metabolism.

Fifty grams lactose. The results of the series of five experiments with the ingestion of 50 gm. of lactose are shown in figure 5. The rise in R.Q. was not so great with the lactose as with the mixture of its two components, 25 gm. of galactose and 25 gm. of glucose, and the fall is slightly more delayed. Even at the end of the experiment the R.Q. has not reached the pre-ingestion level. In this regard the course of the R.Q. differs from all of the preceding series with the sugars. A probable explanation is that there is a slight delay in the effect of the sugars due to the time required for hydrolysis of lactose. This conforms with the earlier work in this laboratory by Carpenter and Lee ('32 c). It also probably accounts for

the fact that the rise in R.Q. is not so great with lactose as with its equivalent hydrolysis products, namely, 25 gm. of galactose and 25 gm. of glucose. The alveolar CO_2 during the first hour was slightly above the pre-ingestion level, but during the third half-hour there was a definite fall. This is the period of time that corresponds to the beginning of the fall in the R.Q. Therefore this series differs from the two

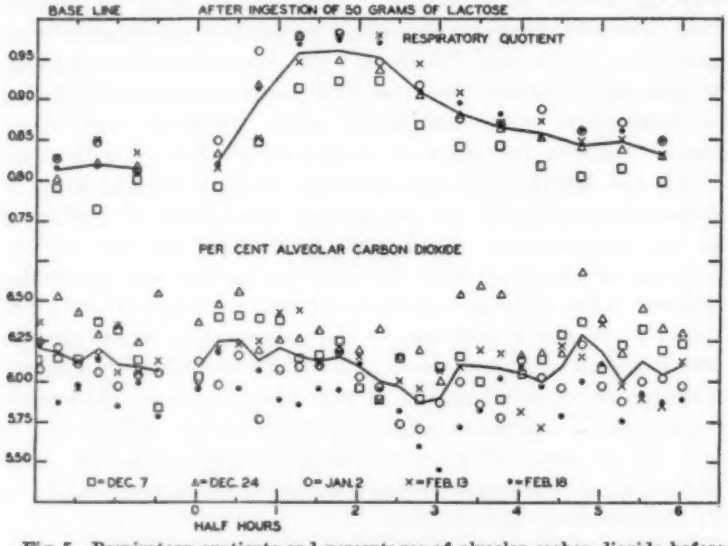

Fig. 5 Respiratory quotients and percentages of alveolar carbon dioxide before and after the ingestion of 50 gm. of lactose in 250 cc. of water. The method of plotting is the same as in figure 1.

preceding series with galactose alone in that a fall in alveolar air does not correspond to the rise in the quotient. Here again the delay may be due to the time required for hydrolysis so that galactose is not set free until later in the experiment than would be found when galactose was given alone or in combination with glucose. After the third half-hour there were irregularities in the course of the alveolar air and in general throughout the whole series. The percentages from one point to another vary considerably. However, whenever

any one group is below the average line it is so for nearly the entire experiment. This is represented in the experiment of February 18th and similarly in the experiment of December 24th. The entire absence of the relation between the R.Q. and alveolar CO_2 in the experiments with lactose is shown in the very low correlation coefficients in table 1. Unlike all the preceding series, with the exception of the control, there is a correlation coefficient with a plus sign as well as others with negative signs. In other words, the coefficients are not consistent among themselves. The coefficients for this series are even less significant than for the preceding series with the equivalent hydrolysis products of 25 gm. each of galactose and glucose.

The preceding series of experiments show that part, at least, of the rise in the R.Q. after the ingestion of galactose must be related to the simultaneous fall in the alveolar carbon dioxide, and that this occurrence is modified by the simultaneous ingestion of another sugar, glucose. Presumably the lowering of the alveolar carbon dioxide is due to the formation of organic acids in the intermediary metabolism of galactose. Judging from previous work on the metabolism of sugars, the acid would be lactic acid. It would have been desirable to determine the lactic acid content of the blood. This, however, was not feasible in these series, and therefore the change in R.Q. due to lactic acid formation cannot be calculated. In an earlier series (Carpenter and Lee, '33 b) with this same subject, the ingestion of glucose or of fructose was not followed by any change in alveolar carbon dioxide, nor was there any change in no-dose control experiments with J.C. (Carpenter and Lee, '33 a). In the latter study, however, there was found with another subject both a lowering of the alveolar carbon dioxide and a rise in the R.Q. in the course of several hours in the no-dose control experiments. An assessment of the parallel changes (Carpenter and Lee, '33 b) in the two physiological factors with that subject showed consistently that for

each 0.10% fall in the alveolar carbon dioxide there was a rise of 0.01 in the R.Q. The application of this method of calculation of the correction of the R.Q. in these series with galactose will result in a lowering of the respiratory quotient of 0.035 in the fifth quarter-hour after the ingestion of 25 gm. of galactose as the average alveolar carbon dioxide was 5.75% in comparison with an average of 6.10% in the pre-ingestion periods. The average R.Q. at this period was 0.90, but in the period preceding this, it was 0.96, so that the maximum fall in alveolar carbon dioxide occurred in the period following the maximum rise in the R.Q. Using the same method of calculation for the experiments with 50 gm. of galactose will result in a maximum lowering of only about 0.030 in the R.Q. in the fourth and seventh quarter-hours after the sugar was given. The average alveolar carbon dioxide was 5.71 and 5.69% in these two periods in comparison with an average of 6.00% in the pre-ingestion periods. The respiratory quotients averaged 0.99 and 0.96 in these two periods, respectively. This method of calculation cannot be applied satisfactorily to the group with 25 gm. each of galactose and glucose combined because the maximum R.Q. occurred in the fourth and fifth quarter-hours after the sugars were given, whereas the most marked lowering of the alveolar carbon dioxide was not until the seventh quarter-hour. In spite of the marked rise in the R.Q. after lactose, there was no significant change in the alveolar carbon dioxide in relation to the changes in the R.Q. Assuming these experiments are of significance in the interpretation of the R.Q. after the ingestion of galactose, the indications are that although organic acids are formed in the intermediary metabolism of galactose, the amounts are not large enough to change very materially the characteristic form of the curve of the R.Q. after the ingestion of this sugar. The amounts formed probably are large enough to explain the occurrence of an R.Q. over unity, but not enough to cause the course of the R.Q. to closely resemble that after the ingestion of glucose.

EXCRETION OF SUGAR IN THE URINE

As a further contribution to the question of the tolerance of galactose the urines were collected and analyzed in these experimental series for the period of time beginning with the arrival of the subject in the morning and ending immediately after the last respiratory exchange measurement. This period therefore included the time during the preliminary rest and the first three quarter-hours of respiratory exchange measurements in addition to the periods after the ingestion of the dose. The average total time covered was about 5 hours. The sugar (reducing substances) was determined by the method of Folin ('26) with the modifications of Folin and

TABLE 2

The excretion of sugar[1] in the urine as affected by the ingestion of galactose, galactose and glucose, and lactose with J.C. (approximately 5 hours)

250 CC. WATER	25 GM. GALACTOSE	50 GM. GALACTOSE	25 GM. GALACTOSE + 25 GM. GLUCOSE	50 GM. LACTOSE
gm.	*gm.*	*gm.*	*gm.*	*gm.*
0.09	0.73	2.11	0.20	0.36
0.08	0.61	1.85	0.21	0.16
0.10	0.38	2.03	0.21	0.17
0.10	0.35	1.53	0.19	0.18
0.08	0.70	1.55	0.72	0.37

[1] Calculated as glucose.

Svedberg ('26). The results are shown in table 2. The average excretion in the control series was only 0.09 gm. calculated as glucose and was very uniform for the series. With 25 and 50 gm. of galactose, there was an average excretion of 0.55 and 1.81 gm. as glucose. Calculated as galactose, the values would be 1.19 and 3.93 gm., respectively. Thus there was a definite and appreciable galactosuria with 25 and 50 gm. of galactose. The same subject in a previous study with galactose (Carpenter and Lee, '32) gave an excretion in about 4½ hours of 0.7, 1.0, 2.2 and 3.1 gm. as galactose with an ingestion of 10, 20, 30 and 40 gm. of galactose, respectively. When 50 gm. of lactose or 25 gm. of galactose and of glucose together were ingested, the average sugar excretion as glucose

was only 0.25 and 0.31 gm. Hydrolysis of the urines after lactose ingestion did not increase significantly the amount of reducing substances. Four of the five experiments with combined galactose and glucose averaged only 0.20 gm. These values are lower than those found with 25 gm. of galactose alone. This is in line with earlier findings on the effect of glucose on excretion of galactose (see p. 591).

TABLE 3

Average changes from the base line in nutrients catabolized and heat production with J.C. after the ingestion of galactose, galactose and glucose, and lactose (3-hour period)

KIND OF EXPERIMENT	CARBOHYDRATES	FAT	HEAT PRODUCTION	
			Average base line	Average change
	gm.	*gm.*	*calories*	*calories*
Control, 250 cc. water	— 2.2±2.4	+0.9±0.9	199	−0.5±1.4
25 gm. galactose	+ 5.5±0.7	−2.2±0.7	197	+1.9±4.0
50 gm. galactose	+15.3±0.5	−5.8±0.3	190	+8.8±3.4
25 gm. galactose + 25 gm. glucose	+12.4±1.7	−5.0±1.2	196	+4.7±4.4
50 gm. lactose	+11.5±2.3	−4.3±1.1	197	+7.0±0.9

METABOLISM OF NUTRIENTS AND HEAT PRODUCTION

Although this investigation was not made to determine particularly the effect of the ingestion of galactose on the catabolism of nutrients, a summary is given in table 3 of the changes in catabolism of carbohydrates and fat, and in the heat production. The calculations have been made in the conventional manner of correcting for the protein on the assumption that the nitrogen excreted in the urine represents the protein catabolized during the period of urine collection. It has also been assumed that the R.Q. was a true metabolic quotient, that is, the result of complete combustion of carbohydrate and fat, and protein except for the residue excreted in the urine. This may seem inconsistent with the finding of a change in the alveolar CO_2, but presumably there is a compensation for the loss in CO_2 so that the R.Q. for the 3 hours

represents the net sums of the total respiratory exchange. Table 3 shows the mean for each series of experiments with the average deviation of the individual experiments from the mean for the group.

The control series does not show any definite effect on either the catabolism of nutrients or the heat production as the average changes are too small and the average deviations too large for the changes to be of significance.

The ingestion of 25 and 50 gm. each of galactose caused increases of 5.5 and 15.3 gm. of carbohydrate catabolized. Earlier studies (Carpenter and Lee, '32 a) gave with this same subject for a 2½-hour period with 20, 30 and 40 gm. of galactose 3.9, 7.5 and 12.8 gm. increase in carbohydrate catabolized. The results in the series here reported are consistent with the earlier studies. The combination of 25 gm. each of galactose and glucose caused an increase of 12.4 gm. of carbohydrate catabolized. An earlier study with this same subject (Carpenter and Lee, '32 c) with 20 gm. each of galactose and glucose resulted in an average increase of 9.2 gm. in 2½ hours. The ingestion of 50 gm. of lactose caused an increase of 11.5 gm. of carbohydrate catabolized in comparison with 12.4 gm. when the equivalent products of hydrolysis were given and 9.3 gm. in the earlier study in 2½ hours after the ingestion of 40 gm. of lactose.

Not only was there an increase in the carbohydrate catabolized, but also a decrease in catabolism of fat. The decrease with 25 gm. of galactose was only 2.2 gm., but it is definite as all five experiments showed a decrease. The decreases were more marked with the other three series of experiments ranging from 4.3 to 5.8 gm. and all of the individual experiments showed decreases.

The average increase in heat production was only 1.9 calories with 25 gm. of galactose and the variability is so large that this increase cannot be considered significant. The increase with 50 gm. averaged 8.8 calories. This value probably does not represent the true increase as there was an increase of only 0.5 calorie in one experiment, and the other four gave

increases ranging from 9.5 to 12.5 with an average of 10.9 calories. The increase in heat production after the combination 25 gm. each of galactose and glucose averaged only 4.7 calories with variations ranging from — 1.0 to + 11.8 calories. The increase with 50 gm. of lactose was more definite at 7.0 calories with the increases ranging from 5.8 to 8.6 calories. In agreement with earlier experience in this laboratory, there was not so good a uniformity in the changes in heat production after the ingestion of sugars as there was in the effect on the catabolism of carbohydrates.

SUMMARY

The respiratory quotient was determined with a human subject in 15-minute periods before and for 3 hours after the ingestion of 250 cc. of water as a control, 25 and 50 gm. each of galactose, 25 gm. of galactose plus 25 gm. of glucose, and 50 gm. of lactose, in 250 cc. of water. Alveolar carbon dioxide was determined simultaneously at 7- to 8-minute intervals during the periods of respiratory exchange. There were five experiments in each group

The ingestion of 25 and of 50 gm. of galactose was accompanied by marked drops in the alveolar carbon dioxide that lasted over 1 hour with 25 gm. and over 1¾ hours with 50 gm. of galactose. In the same periods of time there were marked rises in the respiratory quotients. The correlation coefficients between the alveolar carbon dioxide and the respiratory quotients in the two groups averaged — 0.640 and — 0.666.

With 25 gm. of galactose and of glucose together there was a drop in alveolar carbon dioxide and a rise in respiratory quotient, but the changes in the two factors were not synchronous and the correlation coefficients between the two factors averaged only — 0.424 with a P.E.$_r$ of ± 0.135.

After 50 gm. of lactose there was a fall in the alveolar carbon dioxide for 1½ hours, but the maximum respiratory quotient came earlier, in the third quarter-hour, so that the correlation coefficient between the two factors was only — 0.235. Evidently there was a latency in the effect on the

alveolar air due to the time required for the hydrolysis of the lactose.

Although organic acids were not determined in the blood or urine, the falls in alveolar carbon dioxide accompanying the rises in the respiratory quotient after the ingestion of galactose indicate the formation of acid products in the intermediary metabolism of galactose.

The ascending order of elimination of reducing substances (sugar) in the urine was: control series, 50 gm. lactose, 25 gm. each of glucose and galactose, 25 gm. of galactose, and 50 gm. of galactose.

The increases in the catabolism of carbohydrates and in the heat production in these series agreed in the main with similar series previously carried through in this laboratory.

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EDITORIAL REVIEW

THE NUTRITIONAL AND METABOLIC SIGNIFICANCE OF CERTAIN ORGANIC ACIDS

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The organic acids are widely distributed in many common foods. Citric, malic, tartaric, oxalic and benzoic acids, for example, are present in numerous fruits and vegetables; other less common acids, such as succinic, isocitric, aconitic, tricarballylic, malonic, glyoxylic, salicylic and quinic acids, also occur in some foods. Likewise, the presence of a number of the organic acids in mammalian tissues and body fluids has been repeatedly demonstrated. Questions therefore arise regarding the origin, possible effects, and the ultimate fate of these substances in the organism. Obviously, they may be derived from the preformed acids in ingested food. However, there is convincing evidence that certain acids are endogenous in origin and that they may be formed in the course of the metabolism of the carbohydrates, fats, or proteins. Also, there are increasing indications that certain organic acids are not fortuitous constituents of living matter but that they are involved in definite physiological processes. Although there are other directions in which interest in the organic acids has been focused, the present review must of necessity limit its scope to the more general problems cited above, namely, the occurrence, origin, possible functional significance, and metabolic fate of these compounds. The nutritional and metabolic behavior of 'total' organic acids, as present in certain foods, will be considered first and will be followed by a discussion of several of the more widely studied individual acids known to occur in edible substances.

TOTAL ORGANIC ACIDS

The fact that certain foods upon combustion yield an alkaline ash has long been known. This relationship was determined quantitatively in the comprehensive studies of Sherman and Gettler ('12); the preponderance of base-forming elements was particularly striking in the case of fruits and vegetables. Many of the foods which yielded an alkaline ash on combustion likewise exerted an alkalinizing effect, as indicated by an increase in the pH and a decrease in the titratable acidity of the urine, when ingested by human subjects (Blatherwick, '14). A consistent exception to this general relationship was noted, however, in the case of prunes, plums and cranberries which, although forming an alkaline ash, decreased the pH and increased the titratable acidity of the urine. At that time, the explanation of 'potential alkalinity' of foods offered was that some base was present in the foods combined in the form of organic acid salts, and that during the process of metabolism the organic acid ion was oxidized, largely to bicarbonate. The different behavior of the substances mentioned above was attributed to the presence in these foods of relatively large amounts of benzoic acid, a substance not oxidized in the organism but excreted as hippuric acid.

Satisfactory evidence for the foregoing explanations of the 'potential alkalinity' of foods was lacking until a reliable method for the quantitative determination of organic acids in biological materials was developed. The introduction of the method of Van Slyke and Palmer ('20) for estimating organic acids in urine marked the beginning of the period of significant progress. This method is based on the principle that, after the preliminary removal of phosphates and carbonates with calcium hydroxide, the organic acids of urine may be determined by titration from a pH of 8.0 to 2.7, using suitable indicators. If protein is present, it should be removed before the sample is titrated. Since creatinine, creatine and amino acids are also titrated in this pH range, corrections should be made for these substances, particularly creatinine. The

fact that oxalic, some citric, and perhaps other organic acids are undoubtedly lost during the treatment with calcium hydroxide, appears to be a valid criticism of the Van Slyke and Palmer method. There have appeared numerous modifications of the original method which for the most part involve the extraction of the organic acids from acidified urine prior to titration, changes in the preliminary treatment of the samples to remove interfering substances, or the use of other indicators. Determinations made by the foregoing methods show that the amount of organic acid excreted daily by the normal adult is approximately 6 cc. N/10 acid per kilogram body weight (corrected for creatinine). Methods have also been described for the determination of organic acids in blood by indicator titration, by electrometric titration, and by calculation (subtracting the sum of the anions bicarbonate, chloride, phosphate, sulfate, and the base-combining power of proteins from values for total base of whole blood or plasma). Amounts of organic acids equivalent to 10 to 20 millimols of univalent base per 100 cc. are present in the blood serum of normal adult human subjects.

By means of the foregoing methods, considerable information regarding the fate of ingested organic acids and the behavior of these substances in various physiological and pathological conditions has been obtained. In some of the earlier studies (Blatherwick and Long, '22, '23; Chaney and Blunt, '25), as well as in later ones (Schuck, '34 a), it was shown that the administration to human subjects of organic acids in the form of orange juice led to the excretion of a distinctly alkaline urine and to only a relatively small increase in the amount of organic acids eliminated. These studies demonstrate convincingly the fact that the salts of organic acids as they occur in certain fruit juices yield basic substances in the organism which are excreted in the urine. The striking capacity of the organism to metabolize the organic acids of orange juice was also brought out clearly in studies showing that the acids of as much as 2400 cc. daily of orange juice were almost completely destroyed. Similar

experiments with sour milk showed a comparable utilization of its organic acids. In other studies, however, it was found that the organic acids present in certain other fruits behaved differently than those of orange juice (Blatherwick, '14; Blatherwick and Long, '23; Fellers, Redmon and Parrott, '33). For example, the ingestion of prunes, plums and cranberries was followed by the excretion of a more acid urine. This was attributed to the inability of the organism to oxidize certain aromatic acids present in these fruits.

Likewise, it has been shown that variations occur in the metabolism of the organic acids of other fruits and foods. These studies have included grapes (Pickens and Hetler, '30; Saywell, '32 a; Pratt and Swartout, '33 a; Schuck, '34 a; Clouse, '35), figs and raisins (Saywell, '32 b), tomatoes (Saywell and Lane, '33), pineapples (Miller, '28; Clouse, '35), and loganberries, grapefruit, lemons, apples and sauerkraut (Clouse, '35). Although there is some variation between the results obtained by different investigators, it appears that the juices of the orange and tomato increase the pH of the urine more than do equal amounts of grapes, grapefruit, apples and lemons. However, even in the case of the grape itself, decided variations in response have been observed. This is probably due in part at least to differences in the varieties of grapes used (Saywell, '32 a) and in part perhaps to differences in the amounts employed by various investigators. The fact that grapes increase the pH of urine less than equal amounts of oranges, for example, may be due to the fact that grapes contain a rather large amount of tartaric acid, a compound difficultly oxidized in the animal body.

The question of variations in the total organic acid content of the blood following the ingestion of foods rich in organic acids does not appear to have been studied in detail. It may be pointed out in this connection, however, that no consistent significant alteration in the 'alkali reserve' (plasma CO_2) of the blood has been observed in human subjects following the ingestion of relatively large amounts of organic acids in the form of oranges, prunes and cranberries (Fellers, Redmon

and Parrott, '33; Bischoff, Sansum, Long and Dewar, '34). Few metabolic studies have been made on the organic acids of feces; the presence in the intestine of bacteria, which themselves are known to destroy and to synthesize certain organic acids, makes an interpretation of such studies difficult.

That the organic acids appearing in the urine may not necessarily represent those which have escaped oxidation in the organism is suggested by the work of several investigators (see Schuck, '34 a). For example, the fact that high protein diets augment the excretion of organic acids in the urine has been repeatedly shown (see McLaughlin and Blunt, '23). However, it should be borne in mind that as yet the identity of these acids has not been determined. Likewise, there is other evidence that internal factors may influence organic acid excretion in the urine. Among these may be cited the increased excretion in infant's urine during undernutrition (Utheim, '21), the marked increase found during alkalosis (Goiffon, '25; Fasold, '31), and the decrease following the administration of acids (Fasold, '31). The latter findings have led to the formulation of an hypothesis (Fasold, '31) that organic acids play an important role in acid-base balance regulation. Such factors as exercise, hyperventilation and anoxemia are also known to increase the organic acid content of the blood (Peters, Bulger, Eisenman and Lee, '26) and urine (lactic acid) (Liljestrand and Wilson, '24). Increases in the organic acid content of blood and of urine have been described in pneumonia, diabetes, nephritis, and other pathological conditions. The elevated values in diabetes appear to be due largely to the presence of 'acetone bodies'; the identity of the substances responsible for the rises in the other pathological conditions, however, is still unknown.

Thus it is evident from studies on 'total' organic acids that there are obvious differences in the nutritional significance and in the pathways of metabolism of the various single acids; it now becomes essential to study the behavior of the individual acids themselves.

TARTARIC ACID

Tartaric acid was first prepared as the free acid from 'weinstein' (potassium acid tartrate) by Scheele in 1770 (see Retzius, 1770) by a method described in his first published work. The potassium acid tartrate was converted to the insoluble calcium salt by chalk and free tartaric acid was then produced by treatment with sulfuric acid. Crystalline tartaric acid was prepared by the evaporation of the clear filtrate.

The presence of tartaric acid in a number of common foods is now well established. Of the methods described for the quantitative determination of the acid in biological materials, the potassium acid tartrate and the Kling procedures have been most widely used; they have been tentatively adopted by the American Association of Official Agricultural Chemists. The former method depends on the preliminary precipitation of tartrate as the lead salt, subsequent conversion to and isolation as potassium acid tartrate, and finally titration of this salt with standard alkali. The Kling procedure depends on the permanganate titration of tartrate isolated as calcium racemate, after preliminary precipitation as the lead salt. More recently, a colorimetric method, depending on the formation of a deep red color with meta-vanadate in the presence of tartrate, has been described (Underhill, Peterman and Krause, '31). Under certain conditions the method is said to be specific and reliable. Thus far it has been applied only to the determination of tartrates in urine and other biological material.

Analyses of a few common foods, using the potassium acid tartrate method, have been reported recently (Hartmann and Hillig, '34). Of some twenty-nine fruits and twenty-nine vegetables studied, only the following contained quantities of tartaric acid exceeding 0.014%, an amount believed to be attributable to foreign acid-reacting substances in the cream of tartar precipitate: grape juice, 1.07%; dried lentils, 0.19%; black raspberry, 0.028%; artichoke, 0.020%; avocado, 0.020%; and quince, 0.018%. The outstanding point brought out by analyses thus far reported is the fact that, with the

exception of the grape, only small amounts of tartaric acid are present in the common foods examined.

The question of the fate of tartaric acid entering the organism has been studied by a number of investigators with somewhat conflicting results. This appears to be due, in part at least, both to fallacies in the methods employed and to species differences in the metabolism of the compound. The consistent findings of the early investigators (see Finkle, '33) that only 10 to 40% of tartaric acid administered orally (as the sodium salt, usually) was recovered in the urine, was interpreted as indicating that this acid is largely oxidized in the animal organism. However, subsequent studies in which tartrate has been administered parenterally to different species, including man (Simpson, '25; Underhill, Leonard, Gross and Jaleski, '31; Underhill, Peterman, Jaleski and Leonard, '31; Finkle, '33), have shown conclusively that this belief is entirely erroneous, since 90% or more of the injected compound can be recovered in the urine. Indeed, according to one report (Underhill, Leonard, Gross and Jaleski, '31) even orally administered tartrate is excreted almost quantitatively in the urine in the case of the rabbit, dog and rat.

That the foregoing apparent discrepancy in results is due largely to species differences in the metabolism of tartaric acid is now recognized. In contrast to the above findings in the rabbit, dog and rat, only 20% or less of the tartrate administered orally to guinea pigs and to man was eliminated in the urine (Underhill, Leonard, Gross and Jaleski, '31; Underhill, Peterman, Jaleski and Leonard, '31). No extra tartaric acid appeared in the feces. Three possible explanations for these findings may be advanced: that the ingested tartrate was not absorbed and was destroyed by bacteria or by digestive secretions in the gastrointestinal tract; that it was absorbed and stored in the tissues; that it was oxidized in the organism. It appears unlikely that either of the last two hypotheses is tenable in view of the fact that appreciable storage of tartrate administered parenterally to these species does not occur and that respiratory metabolism studies

(Underhill, Peterman, Jaleski and Leonard, '31) give no indication that oral tartrate is oxidized either by the guinea pig or by man. On the other hand, the evidence appears convincing that considerable amounts of tartrate may be destroyed in the intestinal tract of the rabbit and of man by the action of bacteria (Simpson, '25; Underhill, Peterman, Jaleski and Leonard, '31; Pratt and Swartout, '33 b). That the decomposition is brought about by the action of bacteria rather than by that of some intestinal secretion is indicated by the fact that bile, pancreatin, the contents of the small intestine and Berkefeldt filtrates of fecal material (Underhill, Peterman, Jaleski and Leonard, '31; Pratt and Swartout, '33 b) exert little or no effect on tartrates, whereas fecal material or cultures of fecal organisms alone produce a rapid and complete destruction of the salt. These investigations also obviously indicate that the decomposition of tartrates occurs in the large intestine.

The general observation that foods rich in tartrates, as grapes and grape products, exert a mild 'alkalinizing effect' when ingested in sufficient amounts was discussed earlier. This has been attributed by one group of investigators (Pratt and Swartout, '33 b) to the absorption from the intestine of alkaline decomposition products of tartrates. Possible support for this explanation is given by the fact that the medium in which tartrate had been decomposed by cultures of fecal bacteria became distinctly alkaline. There remains, however, the possibility that the potential alkalinity of grapes may be due to the presence in the grape of substances other than tartrates.

The fact that tartrates may produce nephritis characterized by marked degenerative changes in the renal tubules, regardless of the mode of administration, has been adequately demonstrated in several species (see Rose, '24–'25). The point should be brought out, however, that the production of nephrotoxic effects requires the administration of amounts of tartrate above a fairly definite critical level. In the dog (Underhill, Leonard, Gross and Jaleski, '31) this level appears to be at least 400 mg. of tartaric acid per kilogram body

weight, administered orally. No detectable renal damage occurred in dogs fed smaller amounts than this. Changes in the character of the diet (low calcium, high calcium, high carbohydrate, and high fat) or fasting did not appear to appreciably influence the nephrotoxic effect of tartrate in dogs. According to the same investigators, the rabbit appears to be somewhat more susceptible to the nephrotoxic effects of tartrate than is the dog, doses of 100 mg. per kilogram body weight producing renal damage. While it is recognized that the foregoing amounts required to produce renal damage in experimental animals are far in excess of those which could possibly be ingested in even large quantities of food relatively rich in tartrates, these findings nevertheless indicate the necessity of caution in using tartrates in large or repeated amounts.

MALIC ACID

Another of the organic acids of importance from the point of view of nutrition is malic acid. The ubiquitous distribution of this acid and its salts in nature, the ease of production in large quantities and the promise of greater usefulness in food processing have increased the attention devoted to this substance. Furthermore, the newer studies on intermediary metabolism carried out with isolated tissue indicate that malic acid plays a part in the chemical changes taking place in the body. Scheele (1785 a) discovered malic acid in the juice of unripe apples and berries. Braconnot, in 1808, obtained malic acid from the leaves of euphorbia and its presence in tobacco leaves was demonstrated by Vauquelin the following year. It was first found in the unripe berries of the mountain ash by Liebig in 1833 and for many years this was an important source of the compound.

Liebig (1833) noted the fact that the conventional methods for determining organic acids in plants could not be used for malic acid. According to Hartmann and Hillig ('32) no specific precipitant is known which can serve in the isolation and estimation of the acid. Most of the methods employed

take advantage of the fact that, when uranium acetate or nitrate is added to solutions of malic acid, the optical rotation is markedly augmented, the gross increase being proportional to the quantity of the acid present. The great difficulty has been to secure a solution of malic acid free from the many accompanying constituents of the fruit and plant juices, such as pectin, sugars and other organic acids. The pectin is removed with strong alcohol and the di- and tricarboxylic acids as well as the tannins separated from the sugars and other acids by lead acetate. Tartaric acid is removed as potassium acid tartrate and the other acids by precipitation with tribasic lead acetate. After concentrating, the filtrate is saturated with uranium acetate and the malic acid determined polarimetrically.

A large number of analyses upon fruits and plant tissue have been carried out by Franzen and his co-workers (see Franzen and Schumacher, '21) by precipitating the acids as the lead or barium salts and subsequent fractional distillation of the ethyl esters under reduced pressure. Although this method is well adapted to the identification of organic acids, the large amount of material required for the analyses is a drawback. From this point of view alone, the recent method of Pucher, Vickery and Wakeman ('34) represents a distinct advantage. In this procedure the malic acid is converted by permanganate and potassium bromide into a volatile product which is distilled with steam into diphenylhydrazine in acid solution. The insoluble condensation product, after solution in pyridine, yields a blue color with alkali, which color is proportional to the amount of malic acid originally used. Thus with samples containing as little as 0.2 mg., the recovery of malic acid varied from 96 to 106%.

The extremely wide distribution of malic acid and its salts in plants and plant products is now recognized. Thus, it is found frequently along with other acids, in sugar beets, currants, rhubarb, alfalfa and maple sap, the calcium salt being known in the latter case as 'maple sand.' Hartmann and Hillig ('34) have presented analyses for malic acid in a

large number of common fruits and vegetables. The values in fresh fruits range from 2.48% in Damson plums, 1.59% in quinces and 1.02% in crab apples to as little as 0.16% in strawberries and 0.08% in grapefruit. Of the common varieties of apples the early Yellow Transparent has 0.97% malic acid and the popular Delicious only 0.27%. The vegetables including roots, leaves, seeds and fruits are notably poor in malic acid, as 'acid' a vegetable as the tomato containing only some 0.05%. As might be expected, fruit jellies and jams contain measurable quantities of malic acid (Hartmann and Hillig, '32) as does molasses from sugar cane. According to Steuart ('35) malic acid disappears from apple cider within 10 weeks giving rise to lactic and acetic acids.

Little definite information appears in the literature regarding the specific effects of malic acid in the diet or its behavior in the animal body. The metabolism of organic food acids in general has been discussed earlier in this review; inasmuch as malic acid accounts for a large part of the total acidity in many fruits and vegetables, the discussion of the metabolism of the total organic acids probably applies fairly closely to malic acid itself. Ohta ('12) using an adaptation of the uranyl acetate-polarimetric method for the estimation of malic acid in urine, found that in both the rabbit and the dog a very large proportion of the quantity given by mouth or subcutaneously was destroyed. Sodium malate produces marked gastric irritation according to Underhill and Pack ('25). These investigators gave 1 gm. sodium acid malate per kilogram body weight to fasting dogs and noted prompt and definite reduction in the titratable acidity and a decrease of the ammonia in the urine. These responses are obviously associated with the acid-base balance and indicate that the malate was oxidized most likely to bicarbonate.

Malic acid appears to be involved in carbohydrate metabolism in the animal body as well as in plants. When the sodium salt was administered either subcutaneously or by mouth to phlorhizinized dogs, extra glucose was excreted in the urine and the D:N ratio indicated that it did not arise

from accelerated protein catabolism (Ringer, Frankel and Jonas, '13). Again Ponsford and Smedley-Maclean ('32) showed that in rats fed a carbohydrate-poor diet, ammonium malate per os produced an unmistakable increase in liver glycogen. In contrast to the decided nephrotoxicity of tartaric acid, l-malic acid is innocuous and the d-form only slightly toxic (Rose, '24–'25).

From the available evidence, it appears that, like citrates but in contrast to tartrates, malates are readily metabolized in the body; these acid salts may thus be regarded as potential alkalies in nutrition.

CITRIC ACID

The presence in certain foods of the acid now called citric acid was known to Scheele who isolated the free acid from lemon juice in 1784. The substance was prepared by precipitation of the calcium salt from hot aqueous solution and subsequent crystallization of the compound from weak sulfuric acid solution. Since then the presence of citric acid in varying amounts has been reported in numerous plant substances.

The recent expansion of knowledge regarding the behavior of citric acid in the animal organism has been a direct result of the development of reliable methods for the quantitative determination of this substance. Two general types of methods for the estimation of citric acid are now in use, the 'enzyme' method and methods based on the Stahre reaction (1895). The enzyme method (see Thunberg, '29; Östberg, '31; Kuyper and Mattill, '33) is based on the fact that the enzyme, citric acid dehydrogenase, in the presence of a hydrogen acceptor, methylene blue, catalyzes the liberation of hydrogen from citric acid at a rate proportional to the concentration of citric acid. By determining the rate of decolorization of the methylene blue under standardized conditions, it is possible to determine quantitatively amounts of citric acid as small as 0.2 mg. per 100 cc. This method has the advantages of sensitivity to extremely small amounts of citric

acid and of specificity, although isocitric acid is also dehydrogenated under the same conditions. However, the applicability of the method is limited, as are many methods of this type, because of the extreme sensitivity of the enzyme to variations in the composition of the reaction medium.

Probably the most reliable methods for the determination of citric acid at the present time are those based on the Stahre reaction. The principle of these methods is the oxidation of citric acid in the presence of bromine to an insoluble compound, pentabromacetone, which may be determined by weighing, by titration of its bromine, or by a colorimetric method in which pentabromacetone treated with sodium sulfide forms a yellow-orange colored complex. The reaction is remarkably specific, such closely related compounds as isocitric, aconitic, and tricarballylic acids failing to form pentabromacetone under the same conditions. The few known interfering substances may be removed by preliminary boiling in acid solution or by precipitation with bromine water. The final measurement of the pentabromacetone formed may be made gravimetrically if amounts of citric acid exceeding 50 mg. are present in the samples, as in the case of foods (see Hartmann and Hillig, '34). The titrimetric procedure (see Pucher, Vickery and Leavenworth, '34) may be used if the amount of citric acid in the sample is between 1 and 20 mg., and the colorimetric technic (see Pucher, Sherman and Vickery, '36) is satisfactory for the determination of amounts varying from 0.1 to 1.0 mg. The latter method has proved reliable in the hands of the present authors for the determination of the citric acid content of blood, urine and various tissues.

Using the gravimetric pentabromacetone procedure, Hartmann and Hillig ('34) have reported values for the citric acid content of a number of fruits, vegetables, and miscellaneous foods. Of the fruits, lemon juice contained the highest concentration of citric acid, 6.08%, while only small amounts were found in plums, peaches and apples. Whole oranges and grapefruit contained 0.92 and 1.33%, respectively. Of

the vegetables studied, lima beans, kale, white potatoes and tomatoes were found to contain the largest amounts of citric acid, 0.35 to 0.65%. Small quantities are found in cocoa, malt and wheat germ, whereas certain sea foods, as oysters, clams, crabs, shrimps and scallops, contain almost none. Dried brewer's yeast contains approximately 0.3% citric acid (Sherman, Mendel and Smith, '36 a).

The presence of citric acid in the animal organism was first described by Soxhlet and Henkel in 1888. The crystalline compound was isolated from cow's milk by precipitation of the calcium salt from hot deproteinized whey, and subsequent conversion of the salt to the free acid which was then extracted with ether and crystallized. Since then citric acid has been found in the milk of several species, including that of man. The amount present is surprisingly large, 1.0 to 4.0 gm. per liter (see Östberg, '31); however, colostrum apparently contains no citric acid (Jerlov, '29). The presence of this acid in normal human urine was reported first by Amberg and McClure ('17) and recently the crystalline compound has been prepared from human urine (Fasold, '30). The amount excreted daily by man varies from 0.2 to 1.0 gm. (see Östberg, '31; Kuyper and Mattill, '33; Boothby and Adams, '34; Pucher, Sherman and Vickery, '36). The dog excretes 5 to 20 mg. daily (Pucher, Sherman and Vickery, '36; Orten and Smith, '37) and the rat, 0.3 to 0.6 mg. (1.0 to 2.0 mg. per kilogram body weight) daily (Pucher, Sherman and Vickery, '36). Citric acid is present in the urine of the newborn infant (Lennér, '34; Boothby and Adams, '34). The presence of this substance in almost every other body fluid, and in many tissues has also been described. The following approximate amounts, expressed as milligrams per cent unless otherwise stated, have been reported: sweat, 0.1 to 0.2 (Scherstén, '36); aqueous and vitreous humor of the eye, 3.0 (Grönvald, '36); spermatic fluid, 1.0 to 4.0 (Scherstén, '36); cerebrospinal fluid, 5.0 (Thunberg, '29; Boothby and Adams, '34); saliva, 1.0 (Pucher, Sherman and Vickery, '36); whole blood, 1 to 2 (Östberg, '31; Pucher, Sherman and Vickery, '36); blood cells,

0.5 to 1.2 and blood serum, 0.7 to 1.8 (Pucher, Sherman and Vickery, '36); muscle, liver, kidney, spleen, pancreas, and tumor tissue, 0.1 to 3.0 (Pucher, Sherman and Vickery, '36); feces (dog), 0.4 to 0.8 mg. per 24 hours (Pucher, Sherman and Vickery, '36). Two important facts are brought out by these data, the presence of citric acid in all body fluids and tissues examined and the absence of stores of the substance. The significance of these observations will be discussed subsequently.

Questions logically arise regarding the origin of the citric acid found in animal body fluids and tissues. Obviously it may come from the citric acid present in ingested food. As was pointed out in the section on total organic acids, the available evidence indicates that probably 90 to 95% of the organic acids ingested in the form of orange juice are destroyed in the organism, even when relatively large amounts are consumed. Since citric acid is one of the important organic acids in the orange, these findings suggest that citric acid administered by mouth may be almost completely metabolized. Studies in which pure citric acid was given orally to human subjects have shown that this is true (Östberg, '31; Kuyper and Mattill, '33; Boothby and Adams, '34; Schuck, '34 b; Sherman, Mendel and Smith, '36 b). In the dog, large amounts of orally administered citric acid are likewise destroyed, less than 1% of the compound given appearing in the urine and no extra citric acid appearing in the feces (Sherman, Mendel and Smith, '36 b). Similar results have been obtained in rabbits. The possibility that the administered citric acid was not absorbed appears to be precluded by the finding of a prompt and prolonged rise in the level of blood citric acid immediately after the ingestion of the compound. Likewise, the possible importance of destruction by bacteria in the intestine is minimized by the finding (Langecker, '34) that the enzymes and bacteria present in the intestinal tract of the rabbit do not destroy citric acid even after incubation for as long as 9 hours. Therefore, orally

administered citric acid is undoubtedly absorbed and metabolized by the organism. Some may be converted to carbohydrate, inasmuch as there is evidence that citric acid forms glucose in the phlorhizinized dog (Greenwald, '14). Some is probably oxidized in the organism, perhaps chiefly in the liver and in muscle, since these two tissues are known to contain citric acid dehydrogenase (see Reichel and Neeff, '36). Some support to the latter view is given by the observations that fresh liver and muscle tissue of the rabbit catalyze the in vitro destruction of citric acid (Battelli and Stern, '11; Langecker, '34). The involvement of the liver in the destruction of citric acid is further suggested by the finding of a progressive increase in the amount of the substance excreted in the urine following hepatectomy in dogs (Boothby and Adams, '34). Further indication of the marked ability of the organism to metabolize citric acid is the finding in the rabbit and dog that some 60% of the sodium citrate injected intravenously does not appear in the urine (Salant and Wise, '16–'17; Orten and Smith, '37).

If it is true that citric acid ingested in foods is almost completely destroyed, as the available evidence indicates, the presence of rather large amounts of this compound in the urine under certain conditions, to be discussed, must be explained in some other way. For example, in contrast to the results obtained following the ingestion of the free acid, sodium citrate by mouth leads to the appearance of relatively large amounts of citric acid in the urine (Östberg, '31; Schuck, '34 b; Sherman, Mendel and Smith, '36 a). That this difference is due chiefly to the alkali administered is indicated by the fact that equivalent amounts of sodium as sodium bicarbonate produce similar increases in citric acid output (Östberg, '31; Kuyper and Mattill, '33; Boothby and Adams, '34; Schuck, '34 b; Sherman, Mendel and Smith, '36 a). On the other hand, the administration of hydrochloric acid and potentially acidic substances, as ammonium and calcium chloride, cause a significant decrease in the excretion of citric acid (Östberg, '31; Kuyper and Mattill, '33; Boothby and

Adams, '34). Similar fluctuations have been observed in total organic acids after acid and alkali administration, as was pointed out in a previous section. Apparently, this is due largely to changes in the amount of citric acid excreted. This constant finding led Östberg ('31) to propose the theory that citric acid is an important 'physiological acid' in the same sense that ammonia is a 'physiological base.' Thus, during periods of alkalosis, citric acid is formed to combine with the excess alkali whereas during acidosis such production is diminished. Some further support to this hypothesis is given by experiments on normal human subjects; the mild alkalosis following hyperpnea and the 'alkaline tide' following meals are accompanied by an increased excretion of citric acid (Kuyper and Mattill, '33).

If citric acid is to function as a physiological acid, it is obvious that either a reserve supply of the substance must be present in the tissues and available for emergencies or that an efficient mechanism must exist for the synthesis of the compound as it is needed. As was pointed out in a preceding paragraph, there is no evidence at the present time of the existence of significant stores of citric acid in animal tissues, at least of the dog and rabbit. On the other hand, the evidence for the endogenous formation of citric acid in the dog appears conclusive. Citric acid is excreted in increased amounts during periods of total starvation (Boothby and Adams, '34). Even more convincing is the finding that large amounts of citric acid were excreted daily for periods exceeding a month by dogs fed a citrate-low basal diet to which alkali was added (Sherman, Mendel and Smith, '36 a). The total amount of citric acid excreted far exceeded that present in the diet and that estimated in the animal's own tissues and body fluids.

As pointed out by Boothby and Adams ('34), there are also indications that citric acid may be involved in physiological processes other than the maintenance of acid-base balance. In support of this statement they cite the fact that in their experiments, increases in the amount of citric acid appearing

in the urine did not necessarily correspond with increases in pH. Even more convincing evidence has been recently reported; the intravenous injection of certain compounds, to be discussed later, caused marked rises in the excretion of citric acid with only slight increases in urinary pH (Orten and Smith, '37). The amount of citric acid excreted after the injection of these compounds was far greater than the quantity eliminated after the injection of equivalent amounts of alkali, whereas the increase in pH was greatest after the alkali administration. These findings suggest that citric acid may be a normal metabolite.

Inasmuch as it appears that citric acid may be formed in the organism as a normal metabolite or in increased amounts in response to alkali administration, questions arise regarding its precursors in the body. Studies of this problem have thus far yielded rather inconsistent results. According to Boothby and Adams ('34), metabolic degradation products of carbohydrates do not necessarily serve as precursors since increased amounts of citric acid are excreted by the dog during starvation at a time when the non-protein respiratory quotient indicates that carbohydrate as such is not being burned. These investigators lean to the view that endogenous citric acid formation is related in some manner to the metabolism of protein since in several instances a rather close parallelism between fluctuations in the amounts of urea and of citric acid excreted in the urine were observed. On the other hand, von Fürth and his collaborators ('34) suggest that metabolic degradation products of carbohydrates in the animal organism may serve as precursors of citric acid, as is thought to be the case in molds and fungi. The fact that in his hand sodium acetate elicited a greater increase in the excretion of citric acid in the pig than did an equivalent amount of sodium bicarbonate was interpreted as evidence in favor of this view.

Other recent work (Sherman, Mendel and Smith, '36 a), however, does not yield convincing evidence that products derived in the organism from either carbohydrate or protein

necessarily serve as precursors of endogenous citric acid. No significant change in the urinary output of citric acid was observed in dogs fed a citrate-low ration when either the amount of carbohydrate or of protein in the diet was increased. However, when alkali was also administered there was an indication that the increased excretion of citrate was augmented by a carbohydrate-rich diet, whereas no such effect followed the feeding of a protein-rich diet. Experiments of the foregoing types are undoubtedly complicated to some extent by such factors as the formation of the same metabolic degradation products from both carbohydrate and protein, acid-base effects of the foods themselves and, in the case of the experiments of von Fürth, by possible varying degrees of intestinal absorption and destruction of the compounds administered.

Recently the problem has been reinvestigated by a method which appears to be free from the foregoing objections (Orten and Smith, '37). A number of simple metabolites and related compounds were injected intravenously into dogs maintained on a constant amount of a citrate-low basal ration, and the effects on the daily urinary excretion of citric acid and the urinary pH were observed. Of all the compounds studied, five were outstanding in producing an increase in citric acid output, namely, the di-sodium salts of dl-malic, maleic, fumaric, succinic and malonic acids. The response from these compounds far exceeded the typical 'alkali effect,' as shown by the fact that equivalent amounts of base as sodium bicarbonate and certain other compounds produced only a relatively small increase in citric acid excretion. Since the above dicarboxylic acids may be derived from degradation products of carbohydrates (Needham, '32), proteins (Needham, '30), or possibly fats (Clutterbuck and Raper, '25), the foregoing experiments suggest that endogenous citric acid may be formed from metabolic derivatives of any of the three 'proximate principles.' The degree of the conversion of such metabolites apparently varies with both the supply of the precursors available and the demand for citric acid to maintain normal acid-base relationships. It may be pointed out

that the above four-carbon atom dicarboxylic acids are probably involved in other physiological processes, as in tissue respiration, and as intermediate compounds in the formation of citric acid by molds and fungi. The frequent inverse relation between the amounts of malic and citric acids in certain fruits and vegetables (Hartmann and Hillig, '34) is of interest in this connection.

The question of variations of citric acid in the blood and urine of human patients in pathological conditions has received some attention. According to Thunberg ('33), the citric acid content of the blood tends to be lower than normal in bronchopneumonia, tetany, pleuritis and erysipelas, whereas normal values are found in pernicious anemia, bronchial asthma, mammary and hepatic carcinoma, diabetes, Basedow's disease, chronic nephritis, acute polyarthritis, cerebral tumor and certain other conditions. Values somewhat higher than normal were frequently observed in diabetes, acute nephritis, polycythemia vera and Basedow's disease. It should be noted, however, that the changes described were not large nor were they always consistent in any one disease. Likewise, no consistent deviations from the normal have been found in the quantity of citric acid excreted in the urine (Östberg, '31; Boothby and Adams, '34), with the exception of diabetes accompanied with acidosis. In the latter condition, low values were usually found. Frequently, however, the excretion of citric acid was diminished in nephritis, pneumonia, severe anemia and purpura, and sometimes also in cardiac decompensation, epilepsy and tetany. It is evident that further studies of this type are needed before final conclusions can be drawn.

OXALIC ACID

Oxalic acid is one of the earliest known organic acids. Although Scheele (see Nordenskiöld, 1892) had worked with the oxalates for some 15 years, it was not until 1785 that he described his success in crystallizing free oxalic acid (Sauerkleesalzsäure) and his identification of it with the acid obtained by oxidizing sugar with nitric acid (Scheele, 1785 b).

The quantitative determination of oxalic acid is based essentially on the fact that the calcium salt is highly insoluble; in this form it is isolated and subsequently estimated either gravimetrically, by titration with permanganate or by the measurement of the carbon dioxide evolved in the course of the permanganate oxidation. However, the complex nature of plant juices and of animal fluids and tissue extracts requires that preliminary steps be taken to isolate, as far as possible, the oxalic acid prior to the final estimation. Arbenz ('17) extracted the finely divided dry material with 15% hydrochloric acid, removed the oxalic acid from this extract with ether and, after repeated precipitation as the calcium salt, finally ignited the precipitate and weighed the calcium oxide. According to Hartmann and Hillig ('34) this is probably as satisfactory as any method thus far suggested for determining oxalic acid in plant material. For its estimation in urine, oxalic acid is precipitated as the calcium salt; from the solution of this calcium oxalate in hydrochloric acid, oxalic acid is extracted with alcohol and/or ether, precipitated again as the calcium salt and determined by titration or by weighing after ignition (see Dakin, '07).

In the determination of oxalate in blood the proteins are first removed by trichloracetic acid or by sodium sulfate. From the filtrate oxalate is precipitated with calcium, and the calcium oxalate oxidized with an excess of permanganate which is then estimated iodometrically (Merz and Maugeri, '31). All such direct titration methods have been criticized by Thomsen ('35) on the ground that it is impossible to adequately remove interfering substances from the oxalate precipitate. He suggested recourse to exhaustive extraction of the preliminary acidified calcium oxalate precipitate in order to isolate as far as possible the uncontaminated oxalic acid. Values obtained with this procedure are much lower than those heretofore given.

Although oxalic acid as a constituent of natural foods is ordinarily associated particularly with rhubarb, available analyses indicate that this substance is very widely distributed, especially in foods of plant origin. Thus it has been

found in potatoes, beans, spinach, beets, tomatoes, cauliflower, onions, mushrooms and celery root, among the vegetables and in currants, raspberries, grapes, pears and prunes, among the fruits. Oxalic acid has also been reported in meat, liver and kidney and in coffee, cocoa and tea. It is striking that the richest sources of oxalic acid in foods are the leaves of some vegetables, but there are such wide differences in concentration that general statements in this connection are unwarranted. Thus, kale, turnip tops, mustard greens and dandelion leaves have little or no oxalic acid, whereas spinach contains some 0.7%, beet greens 0.8% and New Zealand spinach 1.2%. Rhubarb stems, the part of the plant usually eaten, have approximately 0.25% and are thus seen to be by no means the richest common source of this substance. For a more extensive list of values for oxalic acid in food materials, see Arbenz ('17), Ryder ('30) and Kohman ('34). It is of interest that many of the so-called 'acid' fruits like apples, oranges, tomatoes, pineapples and strawberries are comparatively poor in this food acid.

Oxalic acid and the soluble oxalates are poisonous to animals and to most of the higher plants. This has given rise to apprehension regarding the use of certain oxalate-containing foods (see Kohman, '34). Unlike some of the other organic acids occurring in natural foods, notably citric, succinic and malic acids, oxalic acid is apparently not readily oxidized in the animal body in the course of metabolism. Furthermore, owing to the marked insolubility of the calcium salt, there is a tendency to remove calcium ions from body fluids and tissues when soluble oxalates are present. It is the manifold disturbances throughout the organism caused by this removal of calcium, to which the toxic property of oxalic acid is largely due. Inasmuch as ordinary dietary practice insures at least a modicum of calcium in the food, it appears that the oxalic acid of the food which is originally soluble would be precipitated more or less completely and thus not absorbed from the intestine. From the point of view of toxicity, therefore, there would seem to be little danger from the presence of oxalic acid in food.

It has long been recognized that both blood and urine contain oxalic acid. The normal values available in the literature vary considerably owing, doubtless, to the variation in reliability of the methods employed for the determination. Loeper ('33) stated that blood usually contains less than 10 mg. %; Jürgens and Spehr ('32) found 12.3 mg. % for the fasting dog and 7.4 mg. % for human blood, whereas Thomsen ('35) found only 1.1 mg. % in the blood of rabbits fed with spinach. Suzuki ('34 a) reported the following values in milligrams per cent for the concentration of oxalic acid in the blood of various species: ox, 3.35; pig, 2.95; dog, 1.90; carp, 3.25; sea bass, 3.23; and salmon, 3.27. The level of oxalic acid in the blood appears to be singularly independent of the oxalate content of the food; neither the ingestion of oxalate-rich vegetables nor fasting changes it (Jürgens and Spehr, '32; Athanasiou and Reinwein, '34). The normal values for oxalic acid in human urine range from 9 to 30 mg. per diem. It is of interest that the urine of the dog contains less than does human urine and the same seems to be true for the rabbit, though, as pointed out by Herkel and Koch ('36), it varies with the diet, increasing definitely when oxalate-rich greens are fed.

The constant presence of oxalic acid in blood and urine raises the question of its origin in the body. There seems to be little doubt that soluble oxalates can be absorbed from the intestine; despite the prompt vomiting induced by orally administered oxalic acid, sufficient was absorbed to bring about a sharp rise in the level of oxalate in the blood in experiments of Jürgens and Spehr ('32). Rittmann and Unterrichter ('35) attribute a greater importance to the food as a source of the oxalic acid of the body fluids than has heretofore been done, suggesting that this has not shown in the balance of intake and outgo because of inadequate analyses of the food. Fruits and vegetables in the diet are recognized as important direct sources of oxalic acid due to the oxalate contained therein; succinic, malic and tartaric acids do not give rise to oxalic acid when given by mouth (Jürgens and

Jürgensohn, '34). Although pure proteins do not appear to increase the output of oxalic acid (Salkowski, '00), connective tissue and gelatin have been looked upon as peculiarly effective in inducing an increased excretion of this substance. This observation has been explained by Suzuki ('34 b) by the fact that commercial gelatin contains sufficient oxalic acid to account for the moderate increase in excretion of this substance after the consumption of gelatin. Moreover, neither glycine nor creatine is a precursor of oxalic acid (Dakin, '07; Jürgens and Spehr, '32).

On a diet extremely poor in preformed oxalate or during inanition, this compound continues to be present in the blood and urine. This circumstance has led to the belief that part of the oxalic acid ordinarily appearing in these body fluids is of endogenous origin. The older literature emphasizes the close relation of oxalic acid formation to carbohydrate metabolism and the newer analyses of blood appear to support this conception. Alimentary hyperglycemia, pancreatectomy and clinical diabetes are accompanied by an increase in the level of oxalic acid in the blood as well as in the urine; insulin prevents the oxaluria (DeLucia and Velardi, '29). Loeper ('33) looks upon insulin as the best therapeutic agent for oxalemia. Adrenalin, on the contrary, is said to raise the level of oxalic acid in the blood (Suzuki, '35). The thesis of the interrelation of carbohydrate metabolism and oxalic acid formation is further supported by in vitro studies of simultaneous glycogenolysis and oxalic acid production in tape worms, liver and heart muscle (Loeper, '33).

The influence of individual amino acids on the level of oxalic acid in the blood has been studied by Suzuki ('34 b). Aspartic acid, asparagine and l-amino butyric acid given parenterally to rabbits produced some increase in the level of oxalate in the blood of rabbits, whereas glycine, dl-alanine, l-glutamic acid and d-amino butyric acid had no influence.

A further possible source of oxalic acid is the bacterial activity in the intestine; according to those who consider this an important contribution to the oxalic acid of the body, the

nature of the substrate and the variety of organism present are of significance. A recent study in which Borgström ('36) demonstrated a greater production of oxalic acid in sterile guinea pigs than in normal control animals again raises the question of the importance of intestinal bacteria as a factor in the production of the so-called endogenous oxalic acid. On the other hand, it is not difficult to understand how the activity of these organisms would complicate the interpretation of dietary studies on oxalic acid.

Oxalic acid exerts an influence in nutrition and in physiology in two major directions, both depending largely on its reaction with calcium. This substance may interfere with the normal utilization of calcium. Götting ('09) cites evidence for changes in the bone of young animals indicating resorption of bone salts as a result of the feeding of oxalic acid. According to Kohman and Sanborn ('35) soluble oxalates withdraw calcium from the body as is shown by the excessive loss in the urine. Again, Fincke and Sherman ('35) observed a marked decrease in calcium utilization in growing rats when spinach provided one-half of the dietary calcium over that when kale was similarly used. These investigators indicated that the oxalate in the spinach was responsible for the interference with the availability of the calcium of the experimental ration. According to Eimer and Bartels ('32) cooking has no effect on the oxalate contained in food. In situations where the calcium of a diet reaches levels admittedly below that dictated by approved nutritional practice, cognizance should be taken of the oxalic acid of the food.

A large part of the renal and ureteral calculi examined contain or consist of calcium oxalate; even the smaller crystals of calcium oxalate may produce tissue injury and hematuria (Braithwaite, '30). The question of oxaluria, its origin and control is therefore of considerable clinical interest and, from the foregoing discussion, appears to be rather closely related to nutrition. Although the output of oxalate in the urine is widely believed to increase in tuberculosis, diabetes and icterus and, latterly, there is some indication that in these

conditions the oxalate level in the blood is elevated, there is little convincing evidence that the output of oxalic acid is influenced one way or the other by renal disease or that urolithiasis is accompanied by marked alteration in the ability of the body to metabolize oxalic acid. The presence of oxalate in the urine depends to a large degree upon the amount of this substance in the diet, the acidity of the gastric juice and the calcium content of the food consumed (Herkel and Koch, '36). Whether or not the highly insoluble calcium oxalate remains in solution in the urine seems to depend upon physical factors, such as the action of non-protein protective colloids, upon adventitious organic foci of precipitation as well as upon the concentration of the salt.

AROMATIC ORGANIC ACIDS

It was pointed out in a foregoing section that there is an increase in the acidity of the urine of human subjects following the ingestion of prunes, plums and cranberries rather than the decrease in acidity expected because of the basic ash yielded by these foods. This apparent discrepancy was first explained (Blatherwick, '14) by the fact that considerable amounts of benzoic acid are present in these foods and that this substance is not oxidized in the organism, as are certain other organic acids, but is excreted as hippuric acid. Subsequent studies suggested (Blatherwick and Long, '23), however, that the amounts of benzoic acid present in the foods were too small to account for the observed excretion of hippuric acid in the urine. Further investigations (Kohman and Sanborn, '31) demonstrated that relatively large amounts, 1.0% on a fresh basis, of another aromatic acid, quinic acid, are present in the prune and cranberry. This compound when fed to man (Quick, '31) was found to be changed in the body to benzoic acid or a closely related substance, and then excreted in the urine as hippuric acid.

Questions arise regarding the fate in the animal body of another aromatic acid, salicylic acid, reported to be present in appreciable amounts in the strawberry, raspberry, blackberry, apricot, crabapple, orange, currant, plum, cherry, apple

and concord grape (see Hartmann and Hillig, '34). Few studies appear to have been made regarding the nutritional and metabolic behavior of this acid. However, it has been reported (Quick, '33) that in contrast to benzoic acid, only a small portion of the salicylic acid ingested by human subjects is conjugated with glycine and excreted as 'salicyluric' acid. Most of the administered compound appears to be excreted as the free acid.

The foregoing studies suffice to indicate that the aromatic organic acids now known to occur in significant amounts in foodstuffs are not oxidized in the organism but are either conjugated with glycine or glycuronic acid and excreted or are eliminated as the free acid. The failure of oxidation of these compounds in the organism apparently accounts for the inability of ingested foods rich in aromatic acids to decrease the acidity of the urine.

OTHER ORGANIC ACIDS

The presence of a number of other organic acids in foods commonly ingested by man has been described (see Hartmann and Hillig, '34). Among these may be mentioned lactic acid in the apple, raspberry, cherry and tamarind; succinic acid in the grape, rhubarb, raspberry, cherry and blackberry; malonic acid in barley, oats, wheat and alfalfa; glyoxylic acid in grape juice; isocitric acid in the blackberry; aconitic acid in barley, maize, rye, wheat and oat plants; and tricarballylic acid in barley and maize plants. Of these, lactic and succinic acids appear to play a significant role in the animal organism. The significance of lactic acid in relation to the metabolism of carbohydrates has been widely investigated and this subject has been adequately reviewed elsewhere. Succinic acid is present in muscle (Needham, '32) and in certain other tissues (Thudicum, 1879; Clutterbuck, '27). This substance may be a degradation product of either carbohydrate (Needham, '32), protein (Needham, '30), or fat (Clutterbuck and Raper, '25; Smedley-Maclean and Pearce, '31). The hypothesis that fumaric acid, formed from succinic acid, plays an

important role as a catalytic link in tissue respiration (Annau et al., '35, '36) indicates the current tendency to attach some physiological significance to the four-carbon atom dicarboxylic acids occurring in tissues. It should be added that as yet, however, there is no unanimity of opinion regarding the alleged role of fumaric acid in tissue respiration.

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The relation of the organic acids present in foods to nutrition and metabolism merits further attention. It seems certain that they are not mere waste products of metabolism in either plants or animals and there is convincing evidence that some of these 'food' acids play fundamental though as yet not well-defined roles in some of the physiological processes of the animal body. It seems well established that several of the organic acids commonly regarded as characteristic constituents of plants are synthesized in the animal body and, in some instances, both the conditions of their formation and their precursors are definitely known. The foregoing account emphasizes the necessity for a consideration of the metabolism of the individual organic acids important in nutrition; only by so doing can a conception of their mode of action and integration in the organism be elucidated.

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THE INFLUENCE OF PARATHYROID HORMONE, UREA, SODIUM CHLORIDE, FAT AND OF INTESTINAL ACTIVITY UPON CALCIUM BALANCE [1]

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The problems of calcium deposit and excretion have been extensively studied in recent years. But the problem of calcium absorption from the intestinal tract has not been adequately investigated or explained. The problem is made difficult by the fact that calcium probably exists in the intestine as a fairly insoluble phosphate, made more insoluble because of the alkaline reaction of the intestinal contents. It is hard to understand, theoretically, why such an insoluble compound should be absorbed at all, though it is obvious that this is accomplished fairly efficiently.

Of the factors which may influence calcium absorption, the need of vitamin D (Bauer, Marble, Claflin, '32 a, '32 b; Gargill, Gilligan and Blumgart, '30) appears to be well established. There is some evidence that the reaction of intestinal contents is also of importance (Abrahamson and Miller, '25; Irving, '26; Bergeim, '26) though this is not definitely established. However, other factors must play a part and a search for them resulted in the following observations.

PARATHYROID HORMONE

In metabolic experiments one must think of intestinal contents as outside of the body. From this point of view the

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influence of the parathyroid hormone and of high blood calcium upon fecal calcium can be twofold; for the intestinal tract can act as an area of absorption as well as an area of excretion for body calcium. The great need in the body for calcium in hyperparathyroidism might well stimulate calcium absorption, while the high blood calcium level in this disease might not only interfere with calcium absorption but might also accentuate body calcium elimination into the feces. Let us consider these two effects.

1. Excretion into the intestinal tract might be accentuated. This can be shown only when a low calcium intake has been used—so that the fecal excretion of calcium is distinctly higher than the ingestion. Much has now been published to indicate that the injection of parathyroid extract does not usually increase fecal calcium excretion (Greenwald and Gross, '26; Brehme and György, '27; Stewart and Percival, '27). When parathyroid extract was administered to our previous cases on a low calcium intake, the fecal calcium excretion was shown to be within normal limits (Albright, Bauer, Ropes and Aub, '29). In a recent observation on a patient convalescent from lead intoxication (E.M., tables 1 and 2), with a constant low calcium diet, the fecal calcium in four control periods averaged 505 mg., while during nine periods of parathyroid extract administration, when the blood calcium was elevated to between 12 and 15 mg.%, the fecal calcium averaged 515 mg. per period. In two cases of parathyroid adenoma reported here (table 1), one, G.M., showed a normal fecal calcium excretion, while on a low calcium intake; the other, A.R. (as also Charles M., reported by Bauer, Albright and Aub, '30), was found to have a very low output of calcium in the feces on a similar diet. This lack of influence, however, has not been universally found, for several cases of parathyroid adenoma have been reported in the literature as abnormal in their fecal excretion. Hunter ('29) found an excessive amount of calcium in the stools of one patient, and of the three cases studied by Bulger, Dixon, Barr and Schregardus ('30), two showed an abnormally high output of calcium in the feces.

TABLE 1

Effect of parathyroid hormone on excretion and absorption of calcium (output and intake in 3-day periods)

SUBJECT	PERIOD	DATE	DIET AND MEDICATION PER PERIOD	CALCIUM			SERUM VALUES Ca
				Excretion		Intake	
				Urine	Feces		
				gm.	*gm.*	*gm.*	*mg.%*
E.M. Normal control	I	Sept. 1933 14–16	Low calcium	0.03	0.55	0.30	9.8
	II	17–19	Low calcium	0.08	0.43	0.30	10.0
	III	20–22	Same + 1500 units	0.08	0.52	0.30	11.9
	IV	23–25	parathyroid extract	0.84	0.48	0.30	16.4
	V	26–28	Same + 750 units	1.34	0.61	0.30	17.1
	VI	29–Oct. 1	parathyroid extract	1.13	0.44	0.30	15.3
G.M. Parathyroid adenoma	I	May 1932 18–20	Low calcium	1.95	0.48	0.29	19.4
	II	21–23	Low calcium	2.15	0.50	0.29	...
	III	24–26	High calcium	2.04	4.03	4.64	...
	IV	27–29	High calcium	2.27	3.15	4.63	18.6
	V	30–June 1	High calcium	2.14	3.06	4.62	...
	Parathyroidectomy—June 4, 1932						
	VI	June 12–14	High calcium	0.04	3.59	4.76	8.7
	VII	15–17	High calcium	0.02	3.20	4.76	7.1
	VIII	18–20	High calcium	0.02	3.12	4.76	7.5
	IX	21–23	High calcium	0.02	2.32	4.76	7.5
	Second admission—September 8, 1932						
	XI	Sept. 14–16	High calcium	0.02	1.60	4.76	10.4
	XII	17–19	High calcium	0.02	1.67	4.76	10.4
	XIII	20–22	Low calcium	0.02	0.10	0.29	...
	XIV	23–25	Low calcium	0.01	0.11	0.29	...
	XV	26–28	Low calcium	0.01	0.09	0.29	9.7
A.R. Parathyroid adenoma	II	April 1932 5–7	High calcium	0.99	2.38	5.84	10.7
	III	8–10	High calcium	1.00	2.10	5.84	...
	IV	11–13	High calcium	0.92	1.90	5.84	11.2
	Second admission—October 17, 1932						
	VI	Oct. 1932 21–23	Low calcium	0.62	0.12	0.29	13.6
	VII	24–26	Low calcium	0.75	0.13	0.29	...

TABLE 1—*Continued*

SUBJECT	PERIOD	DATE	DIET AND MEDICATION PER PERIOD	CALCIUM			SERUM VALUES Ca
				Excretion		Intake	
				Urine	Feces		
A.R.				gm.	gm.	gm.	m.%
Parathyroid adenoma	IX	30–Nov. 1	High calcium	1.93	0.66	5.84	...
	X	2–4	High calcium	2.10	1.06	5.84	13.0
	XI	5–7	High calcium	2.08	1.08	5.84	13.6
	Parathyroidectomy—December 2, 1932 Third admission—January 18, 1933						
		1933 Jan.					
	XIII	26–28	Low calcium	0.02	0.30	0.29	9.8
	XIV	29–31	Low calcium	0.02	0.19	0.29	9.9
		Feb.					
	XVI	5– 7	High calcium	0.13	2.08	5.84	...
	XVII	8–10	High calcium	0.19	2.92	5.84	9.8
F.G.		1935 Jan.					
Parathyroid adenoma	I	20–22	Moderate calcium	1.36	1.13	2.20	15.5
	II	23–25	Moderate calcium	1.45	0.99	2.20	14.2
	Parathyroidectomy—February 8, 1935 Second admission—September 14, 1936						
		1936 Sept.					
	VII	18–20	Moderate calcium	0.14	1.70	2.20	10.0
	VIII	21–23	Moderate calcium	0.19	2.02	2.20	9.6

2. Absorption of calcium from the intestinal tract may be influenced. Since the parathyroid has been repeatedly demonstrated to have no influence on body calcium elimination in the feces, any change in fecal excretion produced by parathyroid over-activity during high calcium feeding would obviously be dependent upon variations in calcium absorption. In order to solve this problem, three classical cases of hyperparathyroidism were studied before and after the removal of the parathyroid tumor. The fecal elimination during identical regimes gives the necessary comparison—the periods of low calcium intake establishing as nearly as practicable the intestinal excretion, while the high calcium periods then give an index of calcium absorption.

Methods. Our usual methods previously described (Bauer and Aub, '27), have been used both as regards the diet and the metabolic ward procedure. All the diets were essentially neutral in reaction with constant water and sodium chloride content. The patients here discussed were perfect metabolic subjects, so that no errors occurred during the observations, and they ate their total diet daily so that no corrections for uneaten food had to be made. The case histories and additional metabolic data may be found in papers by Tibbetts and Aub ('37 a, b).

Results. The results of these observations indicate that the parathyroid secretion exerts a relatively minor influence upon intestinal calcium absorption as well as fecal calcium excretion.

In the patient G.M. (fully reported by Tibbetts and Aub, '37 a, b), the first postoperative observation was started 8 days after the operation and 3 days after returning to the full diet. On the high calcium diet, the fecal calcium excretion in the first postoperative periods showed gradually diminishing values, though the average of all four periods fell only about 10% of the preoperative figures in spite of the dramatic drop in blood calcium level. The fecal phosphorus fell an analogous amount in spite of no change in inorganic phosphorus blood level (Tibbetts and Aub, '37 a, b).

Three months later, when the patient was growing rapidly, a new mechanism had obviously come into play, and both urinary and fecal calcium were being avidly retained in spite of higher blood levels above the so-called kidney threshold (Albright and Ellsworth, '29; Aub, Albright, Bauer and Rossmeisl, '32). This is an experience encountered twice before in growing boys who had essentially no disease (Albright, Bauer, Ropes and Aub, '29; Farquharson, Salter, Tibbetts and Aub, '31). The need for calcium could hardly be greater during growth than during the active decalcification of hyperparathyroidism, yet this new mechanism which improves absorption and prevents loss of calcium in the feces developed slowly and was not manifest during the periods of over-activity

of the parathyroids. That it cannot be ascribed to vitamin D seems clear as this patient received 5 drops of viosterol per day for 2 years previous to operation, and only 10 drops daily between the first and second postoperative studies. The two growing boys, previously reported, received no added vitamin D.

With the patient A.R., this investigation is not so clearly defined, as the first observation was made after a series of x-ray therapy to the pelvis. This produced an obvious though temporary fall in serum calcium levels and the calcium excretion at this time is shown in the results of her first observation. These data show that though the urinary excretion is high, the fecal excretion is only a little lower than that found after operation.

However, in the second admission, when the serum calcium had approached the pre-x-ray level (14.4 mg.%), a different situation occurred for the urinary excretion had risen markedly at the expense of only the fecal calcium while the balance remained the same in the two observations.

After the removal of the parathyroid adenoma, there is poorer absorption and a greater excretion of calcium from the intestine, while there is the usual dramatic fall in urinary excretion. This is true both in the high calcium and in the low calcium periods.

The observations on the patient F.G. were exactly analogous. The second observation was made 19 months after the parathyroidectomy was performed. During this period the patient was on a very high calcium intake, and, though there were still some bone cysts present the x-rays demonstrated much more normal bones. On exactly the same diet in both observations, the fecal excretion of calcium and phosphorus is higher in the postoperative periods. Therefore, these two observations indicate that the absorption of calcium from the intestine was greater during the hyperparathyroid state.

In the clear-cut studies of three patients reported here there is better intestinal absorption of calcium during the hyperparathyroid state in the two adult cases and less absorption

in the adolescent boy. It, therefore, appears clear that the effects of removal of a simple adenomatous parathyroid tumor causes inconstant effects upon fecal absorption and excretion. If the parathyroids were directly involved in intestinal absorption of calcium, one would expect the results to be consistent in all three of these classical examples of hyperparathyroidism. The inconstancy of the findings would appear to justify the conclusion that the parathyroid secretion has notably little to do with calcium absorption, but the dramatic changes which occurred in the adolescent boy make it clear that there are other, unknown but very potent factors which may greatly influence fecal calcium absorption.

Other factors which might influence calcium absorption which we have studied include substances which might affect the solubility of molecules in body fluids or substances which influence the bulk and movements of the intestinal contents.

UREA

Of substances which might affect solubility in body fluids, urea seemed very likely to be of value. This decision was reached because Cohn ('32, '35, '36) has shown that, along with amino acids and proteins, urea has a great solvent action on inorganic ions as well as on the dipolar ions of organic compounds. It is also a normal and widely distributed constituent of the body. There is also the evidence produced by Gamble, McKhann, Butler and Tuthill ('34) that the concentration of some urinary constituents can be increased by the addition of urea to the diet. They, however, did not study urinary calcium. It, therefore, seemed possible that increased concentration of urea in body fluids might increase the solubility of calcium and thereby affect the intestinal absorption, blood level and kidney excretion.

Methods. The metabolic methods of study were similar to those in the preceding report except as follows: Urea was given daily, usually as three doses of 10 to 25 gm. each, by mouth. It was dissolved in water, and because of its great thirst-producing influence, water intake during its use was

TABLE 2

Effect of urea ingestion (output and intake in 3-day periods)

SUBJECT	PERIOD	MEDICATION PER PERIOD	VOLUME OF URINE	CALCIUM			PHOSPHORUS			UREA NITROGEN		SERUM		WHOLE BLOOD NPN
				Excretion		Intake	Excretion		Intake	Excretion in urine	Added to diet	Ca	P	
				Urine	Feces		Urine	Feces						
			cc.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
A.D. (normal control)	XV	Control	5420	0.45	0.50	0.32	2.76	0.79	3.17	....	....	11.0	4.2	..
	XVI	Control	6480	0.47	0.31	0.32	2.73	0.54	3.17	....	....	...	...	..
	XVII	Control	5990	0.55	0.35	0.32	2.80	0.66	3.12	34.3	....	...	...	28
	XVIII	90 gm urea	6480	0.62	0.44	0.32	2.48	0.86	3.17	72.3	42.0	...	...	..
	XIX	150 gm. urea	5210	0.67	0.33	0.32	2.59	0.64	3.16	99.3	70.0	...	...	..
	XX	150 gm. urea	7250	0.80	0.35	0.32	2.68	0.73	3.17	102.2	70.0	10.8	4.0	36
	XXI	Control	6520	0.68	0.32	0.32	2.51	0.65	3.17	35.8	....	...	...	..
	XXII	Control	5960	0.66	0.38	0.32	2.51	0.85	3.17	....	....	11.0	4.4	30
C.W. (normal control)	XV	Control	6530	0.33	0.67	0.32	2.65	0.85	3.17	....	....	10.2	4.5	..
	XVI	Control	6900	0.40	0.51	0.32	2.77	0.67	3.17	....	....	...	...	..
	XVII	Control	7130	0.36	0.60	0.32	2.63	0.75	3.16	34.2	....	10.8	4.2	..
	XVIII	90 gm. urea	6980	0.43	0.76	0.32	2.78	0.07	3.17	76.3	42.0	...	...	..
	XIX	150 gm. urea	6340	0.51	0.58	0.32	2.50	0.81	3.17	99.4	70.0	...	...	..
	XX	150 gm. urea	7330	0.57	0.54	0.32	2.36	0.71	3.17	95.8	70.0	10.3	3.8	37
	XXI	Control	6780[1]	0.49	0.61	0.32	2.81	0.83	3.17	40.4	....	...	...	..
	XXII	Control	6890	0.46	0.49	0.32	2.49	0.61	3.17	....	....	10.5	4.4	31
E.M. (normal control) Huntington Hospital No. 33-1159	VIII	750 units parathyroid extract + 225 gm. urea	8910	0.67	0.63	0.31	1.42	0.89	2.23	92.4	105.0	13.9	3.0	75
	XI	750 units parathyroid extract + 225 gm. urea	8480	0.47	0.55	0.31	1.51	0.97	2.23	116.0	105.0	12.9	3.1	76
	X	750 units parathyroid extract	7380	0.43	0.41	0.31	1.68	0.97	2.23	39.8	....	12.7	...	38
	XI	750 units para-	7190	...	0.41	0.31	2.06	0.80	2.23	80.3	....	12.0	3.5	..
	XII	Control	7660	0.23	0.56	0.31	0.57	1.08	2.23	....	....	10.8	...	..
	XIII	Control	6790	0.09	0.48	0.31	1.03	1.11	2.23	....	....	9.8	3.5	..
M.T.	I	Control	3580	0.42	1.20	1.56	0.93	0.52	1.55	14.1	....	13.7	2.4	..

	XI	750 units para-thyroid extract	7190	...	0.41	0.31	2.06	0.20	2.23	20.3	....	12.0	2.5	..
	XII	Control	7660	0.23	0.56	0.31	0.57	1.08	2.23	....	....	10.6	...	..
	XIII	Control	6790	0.09	0.48	0.31	1.03	1.11	2.23	....	....	9.8	3.5	..
M.T. (hyperparathyroidism) Huntington Hospital no. 33-619	I	Control	3580	0.42	1.20	1.56	0.93	0.52	1.55	14.1	....	13.7	2.4	..
	II	Control	2810	0.43	0.86	1.56	0.77	0.43	1.55	10.0	....	...	...	..
	III	225 gm. urea	7000	0.43	0.83	1.56	0.82	0.45	1.55	95.0	105.0	13.9	1.6	57
	IV	140 gm. urea	5690	0.53	1.20	1.56	1.11	0.52	1.55	82.0	65.0	...	...	63
	V	135 gm. urea	4780	0.56	0.85	1.56	1.01	0.56	1.55	69.6	63.0	16.0	...	68
	VI	Control	1880	0.33	0.88	1.16	0.96	0.33	1.03	29.9	....	13.3	1.5	49
	VII	Control	4110	0.42	0.81	1.53	0.74	0.52	1.55	10.2	....	13.0	1.4	33
R.B. Lahey Clinic no. 33022 (hyperparathyroidism)	I	Control	2450	0.56	...	0.18	...	...	...	....	....	14.0	...	..
	II	Control	3110	0.72	...	0.20	...	...	...	11.7	....	14.7	2.2	..
	III	208 gm. urea	7510	0.85	...	0.20	...	...	...	90.4	98.0	...	...	..
	IV	208 gm. urea	6190	0.85	...	0.20	...	...	...	95.0	97.0	15.8	1.8	80
A.R. Huntington Hospital no. 32-291 (after parathyroidectomy)	XVI	Control	3820	0.13	2.08	5.44	3.44	0.91	6.44	....	....	...	...	..
	XVII	Control	4280	0.19	2.92	5.44	3.50	1.20	6.44	45.8	....	10.2	4.4	39
	XVIII	225 gm. urea	6920	0.21	2.59	5.44	3.69	1.17	6.44	156.9	105.0	...	...	..
	XIX	225 gm. urea	6740	0.21	2.24	5.44	3.64	0.89	6.44	146.5	105.0	9.9	4.2	42
E.D. Huntington Hospital no. 33-370 (myxedema)	I	Control	5380	0.37	0.22	0.31	1.42	0.50	1.86	20.6	....	10.0	3.1	..
	II	Control	5140	0.37	0.21	0.31	1.49	0.48	1.86	21.1	....	10.0	...	30
	III	225 gm. urea	6120	0.41	0.31	0.31	1.59	0.86	1.86	84.5	105.0	10.0	...	..
	IV	225 gm. urea	5860	0.43	0.23	0.31	1.28	0.59	1.86	118.3	105.0	...	...	..
	V	225 gm. urea	6200	0.44	0.32	0.31	1.22	0.79	1.86	114.4	105.0	10.2	3.1	..
M.H. Huntington Hospital no.33-146 (carcinoma of thyroid)	I	Control	3780	0.48	1.18	1.20	1.25	0.90	2.44	15.1	....	9.7	4.6	32
	II	Control	3970	0.49	1.24	1.20	1.23	1.01	2.44	15.0	....	...	...	..
	III	150 gm. urea	4800	0.58	1.22	1.20	1.31	1.04	2.44	71.5	70.0	...	...	..
	IV	225 gm. urea	5890	0.59	1.10	1.20	1.30	0.77	2.44	119.9	105.0	...	...	..
	V	225 gm. urea	5800	0.66	1.24	1.20	1.54	0.74	2.44	117.0	105.0	10.0	4.5	50

[1] Two-day specimen calculated for 3-day period.

TABLE 3

Effect of urea ingestion on serum calcium

SUBJECT	DATE	TIME	SERUM		WHOLE BLOOD NPN	REMARKS
			Ca	P		
	1933		*mg./ 100 cc.*	*mg./ 100 cc.*	*mg./ 100 cc.*	
T.P. P.B.B.H. no. 43292 med. hyperthyroidism	July 17	Fasting	9.8	3.5	28	Control
	July 20	Fasting	10.8		33	75 gm. urea on July 18 and 19
	July 24	Fasting	10.5	3.5	33	75 gm. urea daily July 20th to 24th
E.F. P.B.B.H. no. 44094 surg. hyperthyroidism	July 17	Fasting	10.2		34	Control
	July 20	Fasting	11.4		46	75 gm. urea on July 18 and 19
	July 24	Fasting	11.0		41	75 gm. urea daily July 20th to 24th
H.S. M.G.H. no.330547 Hyperthyroidism	July 17	Not fasting	10.0	3.3		Control
	July 21	Fasting	10.0		34	Control
	July 24	Fasting	10.5		33	75 gm. urea daily July 21st to 24th
	July 27	Fasting	10.6		37	75 gm. urea on July 25th and 26th
		1 hour after urea	10.9		75	
H.M. M.G.H. no. 333961 Hyperthyroidism	Dec. 15		11.2		35	Control
	Dec. 16		10.8	4.9	39	Control
	Dec. 17		10.8		38	75 gm. urea daily Dec. 16th to 21st
	Dec. 20		11.2			
	Dec. 22		11.5		39	90 gm. urea daily Dec. 22nd to 26th
	Dec. 23	Fasting	11.3	4.8	38	
		1 hour after 30 gm. urea	11.2	5.0	60	
	Dec. 26		11.4			
	Dec. 27		11.3			Control
	Jan. 3		11.0	4.8		Control

TABLE 3—*Continued*

SUBJECT	DATE	TIME	SERUM		WHOLE BLOOD NPN	REMARKS
			Ca	P		
			mg./ 100 cc.	*mg./ 100 cc.*	*mg./ 100 cc.*	
	July 17	After breakfast	11.1	2.3		Control
	July 19	After breakfast	11.6	2.0	33	Control
	July 20	Fasting	11.5			25 gm. urea night before
F.C. B.C.H. no. 713282 Hyperparathyroidism	July 24	Fasting	11.6	2.0	24	Small doses of urea July 20th to 24th
	July 28	Fasting	12.4	2.3	27[1]	75 gm. urea daily July 25th to 31st
	July 28	45 minutes after urea	12.7		63[1]	
	July 31	Fasting	12.7		24	
		45 minutes after urea	12.9		49	
D.B. M.G.H. no. 334687 idiopathic hypoparathyroidism	1934					Control
	Feb. 6		5.7	7.4		30 gm. urea Feb. 6
	Feb. 7					45 gm. urea Feb. 7
	Feb. 8					60 gm. urea Feb. 8
	Feb. 9	2 hours after urea	6.8	7.7		20 gm. urea Feb. 9

[1] Serum NPN.

not kept constant but was left to the desire of the patient, except in the case of two normal controls.

Results. The observations need but little discussion, for the tables speak for themselves. The data give some evidence of an effect of urea feeding upon blood calcium levels. This is not evident in the normal controls, even when the blood calcium level has already been elevated by the injection of parathyroid extract (E.M., table 2). But in cases with hyperparathyroid adenomas and in hyperthyroidism (which has an abnormally high calcium excretion (Aub, Bauer, Heath and Ropes, '29)), the addition of urea gives an indication in all

cases of a slight increase in blood calcium concentration (tables 2 and 3).[2]

It is clear that urea taken in large quantities by mouth does not markedly increase calcium absorption from the intestine. This is true in the observations with a low as well as with a high calcium content in the diet, and is also true in conditions in which there is a need for increased calcium absorption, such as in hyperparathyroidism (patients E.M. and M.T.), or in recovery after parathyroidectomy (A.R.).

In relation to urinary excretion the evidence points to a definite elevation of calcium excretion during the ingestion of urea, though this is not of great magnitude. This elevation is probably not surprising when one thinks of the huge substrate of calcium available for solution and for continued excretion, if urea increased the solubility in blood. The increase was found even in the two normal controls (table 2) in whom, because of a constant fluid intake, no diuresis occurred. In the other subjects, whose fluid intake was not restricted, there occurred a large increase in urinary volume because of the great thirst produced by urea ingestion. Associated with this diuresis the balance experiments show an elevation of urinary calcium in all observations, of the same magnitude as in the two controls who had no increased urinary output. Taken from the point of view of urinary concentration, the observations indicate the comparative independence of calcium excretion in relationship to urinary volume.

This independence in excretion of calcium is also obvious in M.T. (see table 2 and appended case history). Her bones were so extraordinarily thin that it was only with very great difficulty that x-ray pictures could be obtained. They showed multiple cysts and multiple spontaneous fractures due to prolonged hyperparathyroidism. Because of her severe nausea

[2] We wish to express our gratitude to the Massachusetts General Hospital, Peter Bent Brigham Hospital, the Lahey Clinic, and the Boston City Hospital, for their kind help in making some of these cases available to us. To Doctor Albright we are also grateful, for his laboratory made the chemical analyses on patient D.B.

it was considered unwise to give her the rigid routine low calcium diet. On her moderate calcium diet (520 mg. Ca per day), it can be seen that her calcium output is not abnormally high, for a normal control who received 700 mg. of calcium per day excreted 390 mg. per period in urine and 980 mg. in feces (Farquharson, Salter, Tibbetts and Aub, '31). Here, then, is a patient with proven hyperparathyroidism, who had, as a result, a diminished calcium reserve of truly remarkable degree. Her enormous need for calcium was not accompanied by great retention of calcium from the bowel, which reacted as in a normal individual. While still capable of maintaining a normal fecal excretion and a markedly elevated blood calcium level, there was still some mechanism present which prevented the usual lavish loss of urinary calcium. This independence of blood elevation and urinary excretion has been previously reported in patients with severe nephritis by Albright, Baird, Cope and Bloomberg ('34). But this patient did not appear to have a nephritis adequate to account for this change in excretion. In spite of this protective mechanism which prevented the great loss of urinary calcium usually associated with hyperparathyroidism, the addition of urea to the diet definitely increased both blood calcium and urinary calcium excretion.

It therefore seems clear that the additional ingestion of large amounts of urea has practically no effect on calcium absorption from the bowel. However, it increased urinary calcium excretion in all observations, including a case extraordinarily depleted of calcium. The effect of urea on the blood was negligible in normal controls, but caused an elevation in hyperthyroid and hyperparathyroid patients.

INTESTINAL ACTIVITY

In the previous sections of this paper were reported the influence of the parathyroid hormone on calcium absorption and the effects of urea ingestion on absorption and excretion of calcium. Other factors which might well influence calcium exchange exist in the bulk and rapidity of fecal

elimination, as well as the interrelation with other inorganic salts. The problem can be approached in two ways: either in regard to absorption, or in regard to intestinal excretion. In these studies, the latter approach was chosen and, therefore, the two subjects (normal medical students) were given a diet adequate to their needs except in regard to calcium.

The purposes of these observations were to determine the effects on calcium excretion of:

1) Variations in bulk and fluid of the feces. 2) The effect of a high fat diet, which, by the formation of calcium soaps, might increase fecal calcium. 3) The effect of urea—already discussed. 4) The influence of large amounts of another physiological salt—sodium chloride.

The two medical students were perfect subjects, and the results are given in full in order to be available as reference for future investigations in this field. Blood calcium and phosphorus values did not show significant changes and are not recorded.

It is to be expected that diarrhea produced by cascara would increase the total base excretion in the feces, and this is accompanied by a similar fall in urinary excretion. But this increased fecal total base excretion is probably largely sodium, for voluntary constipation (periods I–III) and diarrhea (periods IV–VII), and the addition of agar (periods XXIII–XXVI) to the diet caused no significant variation in either fecal or urinary calcium in spite of the great shifts in the wet weight of the feces. Just as calcium excretion appeared independent of urinary diuresis, so here it is independent of fecal volume.

The addition of fat to the diet (approximately 200 gm. per day in the form of butter fat and olive oil) also caused no increased calcium excretion in the feces (periods XII–XIV). The high fecal calcium found in steatorrhea, therefore, represents a wide divergence from the normal inasmuch as we were unable to reproduce it in our normal controls. This period of high fat feeding, however, caused a slight elevation

TABLE 4

A.D. (normal control) (output and intake in 3-day periods)

TABLE 4

A.D. (normal control) (output and intake in 5-day periods)

LOW CALCIUM DIET THROUGHOUT TREATMENT (PER PERIOD)	PERIOD[1]	TOTAL VOLUME OF URINE	WEIGHT OF WET STOOLS	CALCULATED BASES IN DIET[2]	TITRATABLE ACIDITY + AMMONIA IN URINE	NITROGEN		CALCIUM			PHOSPHORUS			TOTAL BASE			CHLORIDE		SERUM VALUES	
						Excretion urine	Intake	Excretion Urine	Excretion Feces	Intake[3]	Excretion Urine	Excretion Feces	Intake[3]	Excretion Urine	Excretion Feces	Intake[3]	Excretion urine	Intake[3]	Ca	P_i
		cc.	*gm.*	*cc. N/10*	*cc. N/10*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*mg.*	*mg.*
Voluntary constipation	I	5870	274	—195	1606	40.8	43.2	0.47	0.45	0.31	2.23	0.66	3.30	4680	910	5199	2340	1870		
	II	5190	153	—195	1426	39.8	43.2	0.47	0.33	0.31	2.20	0.54	3.30	4320	685	5199	2270	1870		
	III	6230	237	—195	1405	39.7	43.2	0.47	0.46	0.31	2.50	0.85	3.30	4250	1620	5199	2580	1870	10.4	4.0
20 cc. cascara	IV	5500	681	—195	1526	38.2	43.2	0.46	0.52	0.31	2.27	0.95	3.30	4100	990	5445	2360	1870		
33 cc. cascara	V	4570	680	—195	1539	36.0	43.2	0.45	0.43	0.31	1.94	0.72	3.30	3330	1260	5605	1755	1870		
61 cc. cascara	VI	5470	884	—195	1643	35.5	43.2	0.53	0.44	0.31	2.34	0.72	3.30	4100	1730	5949	2690	1870		
71 cc. cascara	VII	5710	1316	—195	1783	36.7	43.2	0.57	0.51	0.31	2.34	0.80	3.30	3520	2740	6069	2160	1870		
Control	VIII	5710	344	—195	1755	38.8	43.2	0.47	0.34	0.31	2.44	0.54	3.30	3480	870	5199	2020	1870		
	IX	5990	398	—195	1347	40.2	43.2	0.49	0.44	0.31	2.24	0.69	3.30	4240	1090	5199	2300	1870	10.5	4.0
	X	5500	496	—139	1889	40.6	37.2	0.43	0.37	0.27	2.70	0.66	2.86	3080	990	4487	1100	1640		
	XI	6570	426	—195	1410	39.4	43.2	0.52	0.44	0.31	2.34	0.75	3.30	4050	1250	5199	1815	1870	10.5	
500 gm. added fat	XII	4940	328	—36	1415	40.4	43.3	0.40	0.43	0.30	2.30	0.60	3.20	3100	980	5146	1105	1870		
600 gm. added fat	XIII	4770	544	—72	1691	37.5	43.0	0.39	0.74	0.31	2.02	0.93	3.11	2790	1580	5112	990	1870		
750 gm. added fat	XIV	5935	378	—72	1749	35.8	43.2	0.47	0.50	0.31	2.36	0.58	3.11	3420	1070	5112	1520	1870	11.0	4.2
15 gm. Merck's agar	XXIII	6180	450	—143	1185	34.6	35.1	0.57	0.46	0.26	2.36	0.98	3.34	3880	1090	4205	1560	1380	11.0	4.4
15 gm. Merck's agar	XXIV	5260	262	—195	1186	33.2	35.3	0.52	0.30	0.26	2.10	0.49	2.59	3160	530	4205	1190	1380		
45 gm. Bacto-agar	XXV	4980	446	—243	1222	37.8	43.2	0.66	0.11	0.32	2.15	0.50	2.65	3880	730	5270	1630	1870		
45 gm. Bacto-agar	XXVI	6790	382	—243	1412	40.5	43.2	0.75	0.13	0.32	2.25	0.64	2.89	3800	690	5270	1890	1870		
Control	XXVII	6620	302	—243	1136	41.6	43.2	0.64	0.31	0.32	2.25	0.64	2.89	4500	670	5142	1435	1870	11.8	4.2
+ 30 gm. NaCl	XXVIII	4700	242	—243	1259	40.2	43.2	0.70	0.35	0.32	2.70	0.65	3.35	11500	760	10272	5055	6990		
+ 40 gm. NaCl	XXIX	4930	282	—243	1367	37.8	43.2	0.66	0.35	0.32	2.34	0.64	2.98	9450	770	11982	8995	8690		
+ 45 gm. NaCl	XXX	6800	390	—161	1297	37.2	38.8	0.69	0.31	0.28	2.34	0.51	2.85	10560	690	12257	9135	9350	11.1	4.4
Control	XXXI	5190	246	—243	1654	38.8	43.2	0.51	0.29	0.32	2.18	0.42	2.60	7700	550	5142	1645	1860		

[1] Periods XV to XXII in table 2.

[2] Calculated from Sherman's tables ('27).

[3] Calculated from analyses of diets and individual foodstuffs made in this laboratory.

TABLE 5

C.W. (normal control) (output and intake in 3-day periods)

LOW CALCIUM DIET THROUGHOUT TREATMENT (PER PERIOD)	PERIOD[1]	TOTAL VOLUME OF URINE	WEIGHT OF WET STOOLS	CALCULATED EXCESS BASE IN DIET[2]	TITRATABLE ACIDITY + AMMONIA IN URINE	NITROGEN		CALCIUM			PHOSPHORUS			TOTAL BASE			CHLORIDE		SERUM VALUES	
						Excretion urine	Intake[3]	Excretion		Intake[3]	Excretion		Intake[3]	Excretion		Intake[3]	Excretion urine	Intake[3]	Ca	P
								Urine	Feces		Urine	Feces		Urine	Feces					
		cc.	*gm.*	*cc. N/10*	*cc. N/10*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*cc. N/10*	*mg.*	*mg.*
Voluntary constipation	I	5085	299	—162	1794	40.6	43.2	0.21	0.60	0.32	2.02	0.84	3.31	3340	950	5202	1780	1870		
	II	5760	286	—162	1646	42.9	43.2	0.26	0.64	0.32	2.23	1.05	3.31	4500	1140	5202	1950	1870		
	III	6930	259	—162	1635	44.5	43.2	0.33	0.54	0.32	2.28	0.86	3.31	5300	1050	5202	2160	1870	10.4	4.3
11 cc. cascara	IV	6530	879	—162	1765	42.9	43.2	0.34	0.73	0.32	2.40	1.18	3.31	4100	2000	5337	1880	1870		
24 cc. cascara	V	5800	1065	—162	2228	41.7	43.2	0.30	0.56	0.32	2.24	0.86	3.31	3100	1930	5497	2250	1870		
21 cc. cascara	VI	5360	1106	—162	2348	42.2	43.2	0.35	0.62	0.32	2.30	1.10	3.31	3420	2180	5460	1520	1870		
10 cc. cascara	VII	6660	975	—162	2385	43.8	43.2	0.44	0.58	0.32	2.50	0.80	3.31	3380	1840	5325	2380	1870	10.2	4.4
Control	VIII	6210	458	—162	2012	42.4	43.2	0.44	0.65	0.32	2.40	0.90	3.31	3400	1320	5202	2060	1870		
	IX	6110	431	—162	1732	44.1	43.2	0.38	0.52	0.32	2.58	0.80	3.31	4000	1160	5202	1950	1870		
	X	6420	506	—127	2238	44.7	44.0	0.35	0.69	0.32	2.70	1.14	3.35	2950	1520	5307	1450	1950		
	XI	6920	394	—162	1969	40.7	43.2	0.42	0.44	0.32	2.34	0.71	3.31	3560	1040	5202	1910	1870	10.7	
600 gm. added fat	XII	6370	782	—97	1735	39.9	43.3	0.38	0.71	0.31	1.88	0.98	3.25	2630	1680	5273	1550	1870		
600 gm. added fat	XIII	6590	534	—51	2007	40.6	42.8	0.32	0.62	0.32	2.26	0.73	3.12	2850	1290	5115	1200	1870		
650 gm. added fat	XIV	6420	434	—51	1863	40.5	42.8	0.32	0.56	0.32	2.36	0.65	3.12	3420	1250	5115	1700	1870	10.2	4.5
15 gm. Merck's agar	XXIII	6680	442	—192	1515	36.7	35.7	0.41	0.58	0.27	2.27	0.76	2.60	3340	1120	4205	1580	1380	10.5	4.4
15 gm. Merck's agar	XXIV	6390	440	—227	1536	36.0	36.4	0.38	0.08	0.29	2.00	0.70	2.65	2500	900	4258	1210	1430		
45 gm. Bacto-agar	XXV	5780	556	—222	1776	41.0	43.2	0.48	0.44	0.32	2.00	0.91	3.17	3060	1430	5303	1310	1870		
45 gm. Bacto-agar	XXVI	7640	514	—222	1719	42.8	43.2	0.51	0.63	0.32	2.08	0.77	3.17	3040	1350	5303	1650	1870		
Control	XXVII	7640	310	—222	1521	41.9	43.2	0.48	0.65	0.32	2.22	0.84	3.17	3680	1120	5145	1520	1870	10.4	4.0
30 gm. NaCl	XXVIII	6110	388	—222	1428	42.7	43.2	0.52	0.55	0.32	2.35	0.69	3.17	7340	940	10275	5360	6990		
40 gm. NaCl	XXIX	6070	400	—224	1526	40.9	43.2	0.52	0.57	0.32	2.30	0.82	3.09	9340	1060	11939	8200	8690		
45 gm. NaCl	XXX	7520	242	—222	1527	40.5	43.2	0.53	0.46	0.32	2.27	0.61	3.17	10080	870	12840	9460	9490	10.5	4.4
Control	XXXI	6460	370	—222	2067	45.2	43.2	0.31	0.49	0.32	2.50	0.66	3.17	9200	770	5145	1985	1860		

[1] Periods XV to XXII in table 2.

[2] Calculated from Sherman's tables ('27).

[3] Calculated from analyses of diets and individual foodstuffs made in this laboratory.

of total base excretion in the feces though there was an increased retention of total base because of a greater reduction in urinary excretion.

The addition of sodium chloride to the diet (10 to 15 gm. per day, periods XVIII–XXX) also had minimal effects on the calcium excretion, possibly elevating it a little in the urine. From the total base analyses it is clear that this large addition of sodium chloride to the diet did not increase fecal total base excretion.

It, therefore, appears clear that fecal volume, or a high sodium chloride or fat intake have negligible effects on fecal excretion of calcium and phosphate. A high urea ingestion has a greater effect on urinary calcium excretion than any of these, but that effect is not of large proportions.

DISCUSSION

The evidence introduced in this paper demonstrates the relative stability of calcium absorption and excretion, for this remains remarkably constant in spite of great variations in factors which might be expected to alter its metabolism. The patients suffering from parathyroid over-activity greatly need calcium to counteract the great demands on their bones for this base. Yet there is no evidence that their excessive parathyroid secretion uniformly increases the absorption from the bowel. These observations also indicate that an excellent organic solvent like urea has no apparent influence in aiding calcium absorption nor does voluntary constipation or diarrhea influence its excretion. Yet calcium absorption from the intestine can be markedly improved by some unknown influence (G.M., table 1).

A similar independence is demonstrated in regard to kidney excretion of calcium. It must be recalled that calcium differs from many other bases in the body in that there are vast stores in the bones which can be readily replenished or depleted according to body needs (Bauer, Aub and Albright, '29). In this regard its metabolism resembles that of carbohydrates. One might expect that these large stores of readily

[1] Calculated from analyses of diets and individual foodstuffs made in this laboratory.

available calcium would cause large variations in the amount of its excretion in the urine. But in these observations it is clear that urea increases the calcium excretion only moderately, and this quite independently of whether there is a marked diuresis or not, or whether there is an accompanying rise of serum calcium. Furthermore, in the hyperparathyroid patient who had the smallest calcium stores we have ever seen, a high blood calcium was not accompanied by the usual high urinary excretion. There are, therefore, factors other than kidney threshold alone which influence urinary calcium excretion—evidence for which is also seen in the normal blood levels but elevated excretion found in thyroid and pituitary over-activity (Aub, Bauer, Heath and Ropes, '29; Tibbetts and Aub, '37).

CONCLUSIONS

These observations justify the following conclusions:

1. Parathyroid over-secretion has previously been shown to have no influence on fecal calcium excretion. These observations indicate that it has no consistent effect on intestinal absorption of calcium.

2. An excellent organic solvent like urea does not influence calcium absorption from the intestine. Urea ingestion, however, does elevate the blood calcium level somewhat in exophthalmic goiter and hyperparathyroidism, and increases urinary calcium excretion in all cases. This increased excretion is independent of diuresis.

3. Calcium excretion in normal subjects is independent of voluntary constipation, cascara catharsis, or the ingestion of agar, large amounts of fat or of sodium chloride.

4. Calcium excretion is, therefore, independent of many factors which might be expected to affect it, and in health remains at a remarkably constant level considering the vast available store in the bones.

5. That some factor greatly increases calcium absorption from the intestines is again made obvious in these observations, but its nature is still unknown.

METHODS

The analytical methods used were: Calcium by the method of Fiske and Logan ('31); phosphorus according to Fiske and Subbarow ('25); total base by the Fiske method ('22); chloride by that of Van Slyke ('23); nitrogen by the Kjeldahl; and urea by the urease method described by Folin ('34).

CASE HISTORY

M.T. (C. P. Huntington Memorial Hospital no. 33-619; M.G.H. no. 330046; series, case II; Path. no. 33-2429 and no. 33-4429), female, 53 years of age. For 13 years she had had bone pain which caused her to limp. In the last 4 years she had had operations for 1) a giant-cell tumor of the upper jaw, 2) and one of the nose, 3) uterine suspension, 4) fractures of three bones (3 years ago), 5) curetting cyst of the right tibia ($1\frac{1}{2}$ years ago), and 6) spontaneous fracture of the right femur and of the right tibia and fibula. On physical examination her condition appeared critical due to great asthenia and great anorexia and vomiting.

P.E. A pathetic looking woman with squashed-in torso who has to assume a sitting position because of her fractures and marked upper dorsal spinal kyphosis. She is incapable of moving herself. The only two remaining teeth are very loose and carious. There were a few inconstant rales, and asthmatic breathing in the lungs. Heart appeared normal. Liver edge just palpable. No peripheral edema. Blood pressure 140/75.

Laboratory findings. Urine, with a specific gravity of 1.016, was normal except for a faint trace of albumin, and hyaline and granular casts once. Wassermann, Kahn and Hinton tests were negative. Phenolsulphonphthalein test—intramuscular: first hour, 60 cc. = 15%; second hour 180 cc. = 35%; total = 50%. Non-protein nitrogen = 33 mg.%. Feces were normal. Blood was normal—no anemia. X-ray examination of the whole skeleton showed extensive loss of lime salts and cyst formation in practically all long bones. There were spontaneous fractures of the neck of the left femur and of both the left and right tibiae and fibulae, and of the right clavicle. There was a small substernal tumor mass which pushed the trachea, but not the esophagus, to the right.

After our observations, two operations were performed at the Massachusetts General Hospital by Doctors Churchill and Cope, who found a parathyroidoma composed largely of

chief and giant multinucleated cells. Her recovery has been remarkable. She now feels practically well and is doing a full day's work.

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DIETARY PRODUCTION OF THE SYNDROME OF DEFICIENCY IN VITAMIN B_6[1,2,3]

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ONE FIGURE

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With the knowledge that vitamin G (B_2) is multiple in nature, it has become increasingly important to standardize methods by which the various factors may be determined quantitatively. The present paper describes the steps we have taken and the results we have obtained in an attempt to prepare a diet which will induce vitamin B_6 deficiency with regularity and uniformity.

The Bourquin-Sherman basal diet ('31) for determining vitamin 'G' carries an 80% alcohol extract of whole wheat as a source of vitamin B (B_1). Cereals have been shown to carry a varying but in general a considerable amount of vitamin B_6. About 25% of the vitamin is extracted by the alcohol and from the whole wheat extract prepared in this laboratory (as we have recently reported ('37)) sufficient flavin as well as the 'filtrate factor' of Lepkovsky and Jukes ('35, '36 a, '36 b, '36 c) and Lepkovsky, Jukes and Krause ('36)

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[2] A preliminary report was presented before the ninety-first meeting of the American Chemical Society in Kansas City, April, 1936, and published in the Proc. Soc. Exp. Biol. and Med., vol. 34, p. 296, 1936.

[3] The following materials were generously contributed: cod liver oil by E. R. Squibb and Sons; lactoflavin by the Vitab Products Company, through the courtesy of Mr. G. F. Siemers; vitamin B_1 concentrate by Merck and Company, Inc., through the courtesy of Dr. R. E. Gruber.

can be extracted at the same time to allow a slow but continuous rate of growth. It is interesting to consider, however, that in the early work when the method was standardized, as well as in later work by Sherman and collaborators ('31, '33, '35), by Booher ('33, '34), by Booher et al. ('34), and by Halliday (in East Lansing, Michigan) ('31, '32) dermatitis was induced with a high degree of regularity, particularly when precautions were taken to prevent coprophagy. György ('35) noted that different lots of wheat varied as to their vitamin B_6 value. In this laboratory we have been unable to induce dermatitis on the Bourquin-Sherman diet, due undoubtedly, to a difference in the whole wheat.

With the availability of crystalline or highly potent concentrates of vitamin B (B_1), the whole wheat extract may be eliminated. Theoretically a basal diet deficient in all B factors supplemented by B_1 and flavin with or without the 'filtrate factor' should induce vitamin B_6 deficiency.

The Sherman-Spohn ('23) diet is known to be free of vitamin B and has been considered to be free of all B factors. The composition is: casein (extracted twice with cold 60% alcohol and once with cold 95% alcohol) 18%; Osborne and Mendel salt mixture ('19) 4%; filtered butter fat 8%; cod liver oil 2%; and cornstarch 68%. Our investigations concerned modifications in the carbohydrate, casein and fat content of this diet.

The question of carbohydrate has received much attention and varying results have been reported. Hogan and coworkers ('34, '35, '36 a, '36 b) have found an antidermatitis factor in cornstarch. Bender et al. ('36) demonstrated protection from dermatitis and increased growth with dextrin rather than sucrose in the diet. Chick and co-workers ('35) reported a more regular incidence of dermatitis when maize sugar replaced starch. On the other hand, György ('35), Birch, György and Harris ('35), and Birch and György ('36) have used rice starch extensively in their work on vitamin B_6 and flavin. Jansen, Westenbrink and Boeke ('35) could not induce normal growth on sucrose diets when supplemented

with yeast extract, and preferred rice starch in studies on vitamin B_6. Ariyama ('36) concluded that rats receiving 60% sucrose required, in addition to B, flavin and a concentrate carrying B_6, some factor found to be present in yeast or the alcohol extract of ox liver, lettuce or spinach. He considered the factor was not a member of the B complex. Jackson ('30) found that fatty livers developed in rats receiving an 80% sucrose, fat-free diet supplemented by yeast, wheat germ, cod liver oil, and, in some cases, 10 drops of lard. Sucrose has been used in this laboratory for a number of years when a high degree of purity has been desired in fat-free diets as well as in work on the B vitamins.

Various methods have been used for purification of casein. The English workers have used the 'light white casein' or the 'Glaxo AB' casein without further purification. Supplee and co-workers ('36 a and '36 b) use a casein purified by salt elutions. Elvehjem and co-workers ('36) prepare their casein by precipitation with hydrochloric acid, dissolving in ammonia and reprecipitating with acid. Lepkovsky and Jukes wash commercial casein for 1 week with acidified water followed by one washing with dilute alcohol. Hogan and co-workers ('32, '36 a, '36 b) extract their casein continuously for 1 week with 70% alcohol in a Soxhlet apparatus. We observed that more fluorescent material (flavin?) was extracted by boiling 95% alcohol than by cold 60% alcohol. We therefore extracted the casein several times with hot 95% alcohol until no fluorescence could be demonstrated when the alcohol extract was examined with 'black light,' as described by Supplee and co-workers. This treatment was followed by two extractions with cold 60% and one with cold 95% alcohol.

We made up three diets: 787 was the original Sherman-Spohn diet; 787-A was this diet modified by the inclusion of sugar in place of cornstarch; and 787-B was the diet modified by the inclusion of the more highly purified casein.

In order to evaluate the results we scored the rats with respect to the severity of symptoms as follows: + denotes mild symptoms. The fur, ears and forelegs are blood stained and growth has ceased. ++ denotes a more severe condition.

The ears are swollen, there is a sore area around the nose and mouth, and the feet are red and swollen. At this stage, the animals have begun to lose weight. +++, in this stage the eyes are encrusted, all four feet are raw and the ears are swollen and hard. The nose and mouth are fairly sore. ++++ denotes the final stage. The entire area around the nose and mouth is raw, the feet and forelegs are edematous and sore, the eyes are entirely closed, the ears have sometimes broken off and there is a bloody urine. The animals have lost 10 to 20 gm. in weight.

In spite of the care in the extraction of the casein, we have not been able to induce the denuding on the back and face as described by Hogan, by György and others, as symptomatic of flavin deficiency. Often the abdomen is denuded, but this appears to be due to irritation. We have, however, found denuding of the face in animals which have survived rather longer although they have received flavin. Supplee and coworkers have discussed the difficulty encountered in freeing casein of flavin. They consider the flavin to be bound to casein as a prosthetic group.

EXPERIMENTAL

Twenty-one-day-old female rats weighing 40 to 50 gm. were evenly matched and divided into three groups to test the three diets. A vitamin B concentrate was supplied as a separate supplement. When tested against the Chase and Sherman ('31) vitamin B deficient diet, 200 γ of this material induced a gain of approximately 1.5 gm./day, and this amount was fed to the test rats. (György has found that with too low an intake of B there is a delayed incidence of dermatitis.) Within a few days the rats receiving the sugar diet developed severe diarrhoea which appeared to make them less standard as test animals. It was not possible with the sugar diet to obtain any figure as to food consumption. The animals receiving diets 787-A and 787-B developed dermatitis at about the same time and of about the same degree of severity. Due to these considerations diet 787-B was used extensively for several months. In table 1 are shown the results. The sur-

TABLE 1

A comparison of the incidence of dermatitis, severity of symptoms, and survival periods of rats which received the diets and supplements as described

DIET AND SUPPLEMENTS	NUMBER OF RATS	AGE AT FIRST SYMPTOMS	RANGE	MEAN DEVIATION	S.D.	P.E.	COEFFICIENT OF VARIATION	SEVERITY OF SYMPTOMS, NUMBER OF FLARES	SURVIVAL PERIOD DAYS ON DIET	RANGE	MEAN DEVIATION	S.D.	P.E.	COEFFICIENT OF VARIATION	SURVIVAL PERIOD AGE	RANGE	MEAN DEVIATION	S.D.	P.E.	COEFFICIENT OF VARIATION
		days	*days*				%			*days*				%	*days*	*days*				%
Diet 787 + B_1 only	7	64.7	(54–85)	±34.0				2.9	131	(68–173)	±34.0				152	(89–194)	±34.0			
787-A + B_1 only	5	54.4	(50–57)	± 2.0				3.2	89	(39–120)	±25.2				110	(60–141)	±25.2			
787-B + B_1 only	60	57.3	(32–105)	±11.2	14.7	1.3	25.8	3.3												
Same	38								105	(35–180)	±28.0	33.3	3.6	31.9	125.9	(56–201)	±26.5	33.7	3.6	26.8
+ B_1 and flavin with or without other supplement	17	54.1	(33–69)	±10.8	12.6	2.1	23.2	3.4	86.6	(60–118)	±18.0	20.3	3.3	23.4	107.6	(81–139)	±18.0	20.3	3.3	18.8
Diet 793 + B_1 only	23	54.5	(44–63)	± 4.9	5.8	0.8	10.6	3.7	68.2	(45–89)	± 8.2	10.8	1.5	15.9	89.2	(66–110)	± 8.2	10.8	1.5	12.2
+ B_1 and flavin with or without other supplement	33	56.6	(44–64)	± 5.5	6.3	0.7	11.1	3.5	62.7	(36–87)	±11.1	13.5	1.6	21.5	83.6	(57–108)	±11.1	13.5	1.6	16.1
Diet 794 + B_1 only	10	46.8	(40–55)	± 2.6	3.8	0.8	8.2	3.7	65.1	(55–81)	± 5.1	7.3	1.6	11.2	86.1	(76–102)	± 5.1	7.3	1.6	8.4
+ B_1 and flavin with or without other supplement	13	47.1	(41–55)	± 2.5	1.2	0.2	2.5	3.9	64.4	(50–76)	± 6.9	8.5	1.6	13.2	85.4	(71–97)	± 6.9	8.5	1.6	13.2

vival period of rats receiving diet 787 was very much greater than of rats receiving the other two diets due undoubtedly to difference in the casein. Of sixty rats which received diet 787-B plus B, twenty-two were later used for curative experiments. Survival periods of the remaining thirty-eight animals are shown. Only three rats which received diet 787-B plus B failed to show any symptoms and they were eventually killed. Their results are not included in the table. It is interesting to note that seventeen rats which were used for experimental work and received in addition to vitamin B, flavin with or without a further supplement low in vitamin B_6 survived only 86.6 ± 18.0 days on the diet. They thus showed a shorter survival period than those which received B supplement only, and confirmed the findings of Chick ('35) and co-workers on this point.

In all experimental work rats were given at weaning the basal diet plus vitamin B, and in some cases B and flavin until growth ceased and symptoms of about 1+ degree of severity were induced. This required from 4 to 5 weeks on an average for diet 787-B, and about 3 to 4 weeks for the sugar diets used in later work to be described.

It was still felt that because of its relatively greater purity sugar would be preferable to starch. We therefore investigated the effect of lowering the fat content, in an effort to reduce the incidence of diarrhoea. György, and Bender and co-workers had both reported some fat sparing action with respect to vitamin B_6.

Diets 793 and 794 (the latter carrying casein extracted according to the Hogan technic) were prepared. The composition was as follows: casein 18%, salts 4%, filtered butter fat 3%, cod liver oil 2% and sucrose 73%.

From table 1 it is seen that results with these two diets were closely similar. There was not the large difference between animals receiving additional supplements and those receiving vitamin B only as was seen in the earlier work with diet 787-B. There was 100% incidence of dermatitis of nearly 4+ severity. There were no spontaneous cures. However,

there was considerable diarrhoea, particularly in the later stages.

Lepkovsky, Jukes and Krause ('36 d) find that on their purified diet, supplemented by vitamin B and flavin, most rats die without manifesting symptoms. Addition of the 'filtrate factor' is necessary before dermatitis develops. It would hardly seem that our diet could carry any of the 'filtrate factor.' It is postulated, therefore, that the growth stimulus furnished by this added factor might cause the dermatitis to become severe, in the experiments described by these workers. In a recent paper Elvehjem ('36) and co-workers describe a series of diets used in preliminary work on their liver growth factor. The diets carry starch, dextrin or sugar, and from 7 to 14% fat. Animals given these diets supplemented by vitamin B failed to grow, and the authors state that the rats failed to show any symptoms which might aid in determining the specific deficiency. György found that the average time of appearance of symptoms was about 7 weeks. In these experiments the rats were depleted 21 to 25 days and the greater majority of them, unless protected, died within the next 5 weeks.

It seems, therefore, that diets 793 and 794 can be depended upon for uniform results. If it is desired to obtain food records, diet 787-B can be used.

We cannot explain why we found it possible to induce the specific dermatitis on a cornstarch diet. One of us has visited Doctor Hogan's laboratory, and, as far as it is possible to judge, the dermatitis he induces and cures with wheat germ oil is identical with the conditions we describe. We have not been successful as yet in curing dermatitis with wheat germ oil in this laboratory, but these experiments are still under way.

Itter and co-workers ('35) correlated alopecia with the absence of the sulfhydryl group, and found that feeding of cysteine HCl led to cures. Prunty and Roscoe ('35) showed that purified casein might be deficient in cystine, with the result that the growth rate was lessened. They could not cure

the dermatitis symptomatic of B_6 deficiency by the addition of cystine, however.

In order to ascertain whether or not cystine could be a limiting factor in our results, we prepared a modification of diet 787-B in which 0.25% cystine replaced that amount of starch. A paired feeding experiment was carried out, the animals which received the non-supplemented diet acting as

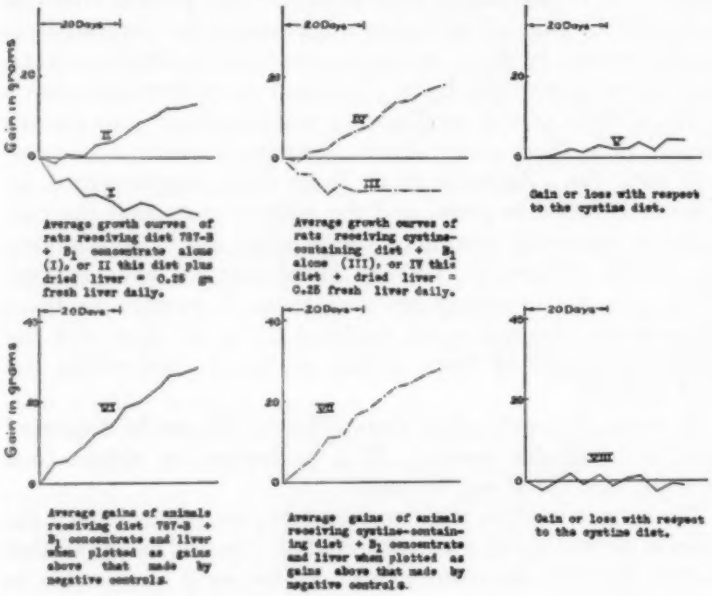
20 Days
Gain in grams
20
0
II
I
Average growth curves of rats receiving diet 787-B + B_1 concentrate alone (I), or II this diet plus dried liver = 0.25 gm fresh liver daily.
IV
III
Average growth curves of rats receiving cystine-containing diet + B_1 alone (III), or IV this diet + dried liver = 0.25 fresh liver daily.
V
Gain or loss with respect to the cystine diet.
40
VI
Average gains of animals receiving diet 787-B + B_1 concentrate and liver when plotted as gains above that made by negative controls.
VII
Average gains of animals receiving cystine-containing diet + B_1 concentrate and liver when plotted as gains above that made by negative controls.
VIII
Gain or loss with respect to the cystine diet.

Figure 1

controls. Prunty and Roscoe had stated that when a low level of vitamin 'B_2' was fed, addition of cystine improved the growth rate. We therefore fed (in addition to vitamin B) dried liver $\simeq$ to 0.25 gm. fresh material to the experimental animals of the two groups. Figure 1 shows the results. There was definitely no increase in growth as a result of the cystine addition. Furthermore, rats which received only vitamin B in addition to the cystine diet developed dermatitis

somewhat earlier than did those of the non-supplemented diet group.

Using the method of Prunty ('33) for determination of cystine we found 0.34%, 0.24% and 0.20% for the cystine content of the unextracted casein, the casein used in diets 787-B and 793, and that used in 794, respectively. Thus the cystine content is lowered but, from our experimental results, it would seem that neither the loss in weight nor the severe dermatitis produced could be due to cystine deficiency.

The occurrence of diarrhoea among the animals in this work appears to be somewhat unique. Doctor Hogan has used a low fat, high sugar diet for some years and has not encountered the phenomenon. Lepkovsky and Jukes ('36 c), using 59% sugar and 10% lard in their diet state that diarrhoea is a characteristic symptom of flavin deficiency. Many of our rats, however, developed the condition while receiving flavin. It seems that further work will be required to answer this question.

We did not use the Page ('32) anti-coprophagy harness in these experiments. Had we done so, it is quite probable that the variations which occurred within groups would have been materially lessened.

CONCLUSIONS

Experiments were carried out in an attempt to develop a basal diet for rats which would induce the syndrome of vitamin B_6 deficiency with regularity and uniformity.

The effects due to the method of extraction of the casein, the type of carbohydrate and the percentage of fat in the diet were studied.

From results with large numbers of rats it is concluded that a diet carrying high sucrose, low fat, and casein extracted with both boiling 95% alcohol and cold 60% alcohol can be depended upon to produce uniform results as to severity of symptoms and length of survival period of rats. A system of scoring the symptoms was developed which permitted quantitative comparison of the animals in the various groups.

Although the method of preparation of the casein lowered its cystine content, this did not appear to be a factor in our results.

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THE FOOD INTAKE OF YOUNG RATS HELD AT NEARLY CONSTANT BODY WEIGHT BY RESTRICTION OF THE DIETARY PROTEIN [1]

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ONE FIGURE

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It is well known that growth in young animals can be retarded or suppressed by restriction of the dietary intake of protein. The morphological changes, especially in the organ weights, under such conditions were studied by Limson and Jackson ('32) and Jackson ('36). The present report deals with the food intake during the latter experiment. The data include the amount of protein (in the form of yeast-wheat germ mixture) required to maintain the body weight, and the corresponding voluntary intake of basal diet when the protein intake is thus restricted.

MATERIAL AND METHODS

The albino rats used in this work are from the standardized colony maintained for nutritional experiments in the Institute of Anatomy. They were originally derived from the Wistar experimental strain. The test rats used in the present study include 35 males and 32 females (with 3 in addition to the 33 males and 31 females reported by Jackson, '36). Large groups of animals are desirable on account of individual variations, as shown for metabolism by Benedict and

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MacLeod ('29). All the rats were weaned and weighed on the twenty-first day of age, when the experiment began.

The test rats were then placed in metal individual cages with wire-net floors, on the protein-deficient diet used in the two preceding studies. The basal diet mixture, fed ad libitum, included sucrose 75%, lard 20%, and salt mixture (McCollum 185) 4.5%. The consumption was measured by weighing the initial amount placed in the cage, and later the residual amount left in the food cups, together with the scattered food carefully collected from a paper sheet placed beneath the floor of the cage. City tap water was also supplied ad libitum from bottles in the cages. The accessories, fed separately to each rat daily, included 2 drops of cod liver oil (Patch), and a variable amount of mixture of equal parts by weight of dried brewer's yeast (Northwestern pure dehydrated) and wheat germ (Russell Miller Milling Co.). As explained by Limson and Jackson, this diet provides adequate nutrition for all the known factors except protein, which is represented by the yeast-wheat germ mixture. This mixture was fed in varied amounts from day to day, regulated so as to hold the body weight as nearly constant as possible during the experimental period of 15 weeks, that is, from 3 to 18 weeks of age.

During the period of growth repression, the test rats were kept in a constant temperature room at about 26°C. (79°F.) with a daily variation of less than 1°C. According to Benedict and MacLeod ('29), this is slightly below the critical temperature for the rat. While in this room, the rats were observed and weighed daily.

OBSERVATIONS

The general appearance of the rats during the experiment was reported by Jackson ('36) and need not be repeated here.

Body weight. As shown in figure 1, it was not possible to hold the body weight entirely constant. A slight loss was unavoidable during the first week of the test, while the rats were becoming adjusted to the new diet. Thus the average of the thirty-five males decreased from the initial weight of 49.0 gm.

to 46.1 gm. at the end of the first week. The loss was soon recovered, and the weight remained fairly constant between 50 and 51 gm. from the sixth week onward. Similarly the average weight of the thirty-two females decreased from 47.2 to 44.2 gm. during the first week, then recovering to a level between 48 and 49 gm. which was maintained from the sixth

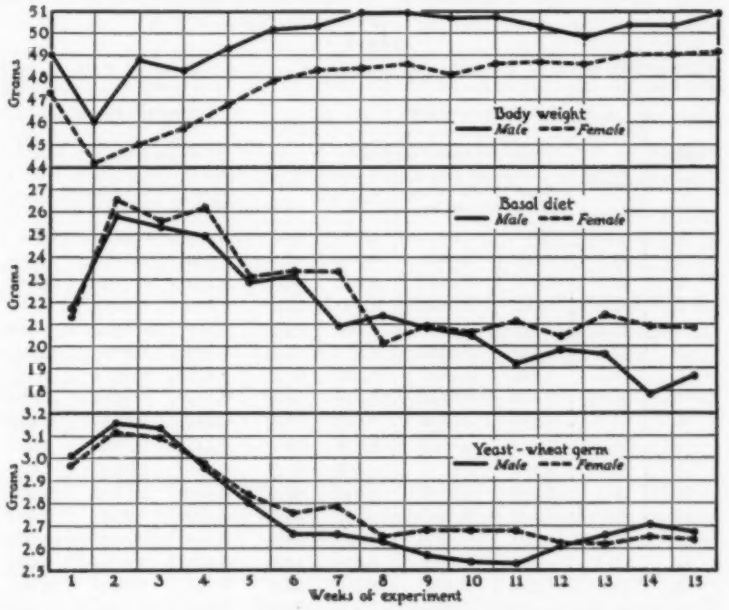

Fig. 1 Curves showing the changes in average body weight for each sex by weeks during the period of the experiment; with corresponding changes in the consumption of basal diet (ad libitum) and of yeast-wheat germ mixture (regulated to hold the body weight nearly constant).

week onward. The figures for body weight for the various weeks in table 1 differ slightly in that they represent the estimated averages for the corresponding weeks, instead of the weights at the beginning or end of the week. The mean of the weekly average male weights (49.81 gm.) was about 2 gm. above that of the females (47.65 gm.). This represents

the average body weight for each sex during the entire experimental period.

Protein requirement. As is evident in figure 1, the amount of the yeast-wheat germ mixture required to maintain the body weight under these conditions showed a characteristic change during the course of the experiment. During the first week (period of adjustment to the diet) the average intake

TABLE 1

Weekly average body weights and intakes of food per gram of body weight

WEEK	THIRTY-FIVE MALES				THIRTY-TWO FEMALES			
	Body weight	Intake per gram body weight			Body weight	Intake per gram body weight		
		Basal diet	Yeast-wheat germ	Total [1]		Basal diet	Yeast-wheat germ	Total [1]
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	47.5	0.456	0.0633	0.534	45.8	0.466	0.0649	0.546
2	47.5	0.542	0.0663	0.623	44.6	0.595	0.0700	0.681
3	48.5	0.522	0.0645	0.600	45.4	0.565	0.0681	0.648
4	48.8	0.511	0.0605	0.586	46.3	0.566	0.0642	0.645
5	49.7	0.459	0.0563	0.529	47.3	0.488	0.0600	0.563
6	50.3	0.462	0.0530	0.529	48.1	0.463	0.0574	0.535
7	50.7	0.413	0.0525	0.479	48.3	0.483	0.0576	0.556
8	51.0	0.419	0.0516	0.485	48.5	0.414	0.0546	0.483
9	50.9	0.409	0.0505	0.473	48.3	0.431	0.0555	0.501
10	50.7	0.404	0.0501	0.468	48.3	0.426	0.0555	0.496
11	50.5	0.381	0.0501	0.445	48.6	0.435	0.0552	0.504
12	50.0	0.396	0.0522	0.462	48.6	0.422	0.0539	0.490
13	50.0	0.391	0.0532	0.458	48.7	0.440	0.0538	0.508
14	50.3	0.356	0.0539	0.424	49.0	0.426	0.0541	0.494
15	50.7	0.368	0.0527	0.435	49.0	0.424	0.0539	0.492
Average	49.81	0.433	0.0554	0.502	47.65	0.470	0.0586	0.543

[1] Total includes the cod liver oil (2 drops daily per rat).

of the mixture per rat was about 3 gm. Beginning with the second week, the consumption decreased steadily from over 3.1 gm. to about 2.7 gm. in the sixth week. This decrease in protein requirement corresponds to the period of slight increase in average body weight. Thereafter, during the period of nearly stationary body weight, the requirement of yeast-wheat germ likewise remained nearly constant at the comparatively low level of 2.6 or 2.7 gm. per week, or slightly

below 0.4 gm. per rat daily. The corresponding data, figured on the basis of the weekly intake per gram rat are shown in table 1. As was noted by Limson and Jackson ('32), the actual protein component probably does not exceed one-third of the yeast-wheat germ mixture.

Another feature of interest is the apparent sex difference in the required protein intake. The difference in the total intake is not striking, as shown in figure 1, although the female intake appears higher during the greater part of the period. When the difference in body weight is taken into account, however, as shown in table 1, the average protein intake per gram rat appears invariably higher in the female. The difference is not very great (average 5.8%) but the constancy makes it probable that it is significant. The usual statistical formula for the probable error of the difference in means is not appropriate here, because we do not have 'normal distribution' of the variates. More complicated methods of measuring the significance of the difference were not attempted. We may, however, conclude that the males are apparently able to maintain their body weight with a relatively lower protein intake.

Basal diet intake. The amount of basal diet voluntarily consumed by the rats runs closely parallel to their restricted protein intake, as shown by the general course of the curves in figure 1. After an initial increase in the second week (following the period of adjustment in the first week), there is a steady decrease in the basal diet consumed during the first half of the experiment, followed by a more nearly stationary period in the second half.

The ratio of the basal diet intake to the yeast-wheat germ mixture during the successive weeks remains remarkably constant. From the data given in table 1, the percentage of yeast-wheat germ intake to basal diet may be calculated for either sex, on the basis of consumption per gram of body weight. The percentage of yeast-wheat germ in comparison with total food intake (basal diet plus yeast-wheat germ and cod liver oil) is slightly lower but similarly constant, averaging close to 11% for each sex (general range 10% to 12%).

While the ratio of protein to voluntary total diet thus appears similar in each sex, there is an apparent sex difference in the amount of basal (and total) diet consumed. As shown in figure 1, the average female intake of basal diet exceeds that of the male in every week excepting the first and eighth. This difference is still more apparent when calculated on the basis of intake per gram body weight, from the data in table 1. The average weekly intake of basal diet per gram body weight in the females (0.470 gm.) exceeds that of the males (0.433 gm.) by 8.5%. The percentage difference for total dietary intake is about the same. Here again, as above mentioned for the yeast-wheat germ, the usual statistical formula for probable error of the difference cannot properly be employed, since this requires a 'normal distribution' of the variates. More complicated statistical methods were not attempted, but even without these the significance of the sex difference can scarcely be doubted.

Dietary calories. The energy value of the various dietary components consumed was calculated at 4 calories per gram for the sucrose and yeast-wheat germ, and 9 calories per gram for the lard and cod liver oil (assuming 2 drops = 0.1 gm.). The changes in energy from week to week necessarily correspond to the weights of the diet consumed. The total dietary intake by the male rats during the 15 weeks' period averages 1807.72 calories per rat. The corresponding amount for the female rats averages 1872.94 calories, or 3.6% greater. Since the body weight of the female rats is lower, the caloric value of their diet consumed per unit of body weight is correspondingly greater. Calculated on a relative basis, the energy value of the total diet consumed averaged 0.346 calorie daily per gram body weight for the male rats, and 0.374 calorie for the females. This amounts to an excess of about 8% for the females, corresponding to the difference in weight of diet consumed during the period.

DISCUSSION

The well-known decrease in the general dietary requirement for young animals during the course of maintenance experiments might be explained in different ways. The most important factor is probably the associated decrease in basal metabolism resulting from inanition. This decrease has recently been shown in fasting rats by Benedict and Fox ('34) and in stunted young rats by Horst, Mendel and Benedict ('34). The latter observed a lowered basal metabolism (heat production) when growth was repressed by low protein diet, as well as during underfeeding tests. They also noted that the lowered metabolism occurs in spite of increased activity. An increase in the spontaneous activity of the stunted rats was likewise evident in the present experiment.

Since in the present investigation the voluntary food intake was reduced in proportion to the limited protein, it is probable that general caloric deficiency may be another important factor in the associated repression of growth. This conclusion is supported by the data of McCay, Crowell and Maynard ('35), who arrested growth by general underfeeding of a somewhat different diet. They found that to maintain the body weight of rats at 50 gm. required 0.286 calorie per gram rat daily for the males and 0.304 calorie for the females. This is not far below my average of 0.346 calorie consumed by the males and 0.374 calorie by the females. My results are therefore in harmony with the conclusion of R. W. Jackson ('29) that a rat eating ad libitum of a diet limited in one essential factor (so as to cause approximate weight maintenance) will ingest very little more energy than can be used advantageously under these conditions.

In experiments extending over a considerable period of time, the age factor may also be contributory in reducing the nutritive requirement. In general, the heat production in young rats appears normally to decrease with age, as shown by Davis and Hastings ('34), Sherman and Campbell ('35), and Sherwood ('36). McCay, Crowell and Maynard ('35) similarly found that at successive stages of greater age and

body weight there is a quite regular decrease in the calory requirement for maintenance per unit of body weight. As is pointed out by Johnson, Hogan and Ashworth ('36), the relative protein requirement likewise diminishes normally with age.

Numerous workers have discovered sex differences in the nutritional requirements of rats. Horst, Mendel and Benedict ('34), observed that, in general, adult male rats have a higher basal metabolism, which is in accordance with the usual finding in other species. However, Davis and Hastings ('34) and Sherwood ('36) could find no appreciable sex difference in basal metabolism, especially in younger rats. Wang ('25) observed a relatively greater food intake in the female rats. Mitchell and Carman ('26) and Morris, Palmer and Kennedy ('33) concluded that the female rat consumes relatively more energy per unit gain in body weight. McCay, Crowell and Maynard ('35) similarly noted that the female rat requires more calories for maintenance. In the absence of any evidence for higher basal metabolism in the female rat, the workers have usually ascribed the higher energy intake of the female to greater activity. Apparently the only direct evidence of such activity was published by Slonaker ('12), whose conclusions were based upon a female rat showing greater activity than the males after the fourth month of life. Limson and Jackson ('32) observed no appreciable sex diference in the intake of food under conditions similar in general to the present experiment, but at ordinary fluctuating room temperatures instead of constant temperature. The present results support the conclusion that the energy requirement is relatively higher in the female, whatever may be the significance of this phenomenon. It furthermore appears that this sex difference applies not only to the total diet, but also to the protein (yeast-wheat germ) requirement for maintenance.

SUMMARY

Thirty-five male and thirty-two female albino rats (Wistar strain) were maintained for 15 weeks at body weight of about 50 gm. by regulated amounts of protein (yeast-wheat germ) intake. A diet otherwise adequate for normal nutrition was fed, including 2 drops of cod liver oil daily with basal diet ad libitum. The room was held at nearly constant temperature (26°C.).

Under these conditions, the required (limited) intake of yeast-wheat germ mixture increased slightly during the first week (period of adjustment). Beginning with the second week, the consumption decreased steadily from an average of over 3.1 gm. per rat to about 2.7 gm. in the sixth week, remaining nearly stationary thereafter.

The corresponding voluntary intake of basal diet ran closely parallel to the limited protein intake, the average weight of yeast-wheat germ remaining nearly constant at about 11% of the total diet (including accessories). The calories of the total diet appeared slightly above the energy requirement for maintenance.

The data indicate a slight but probably significant sex difference in the food intake of the rats under these conditions. The male rat apparently requires relatively less protein (yeast-wheat germ) for maintenance, and shows likewise a relatively smaller intake of the basal and total diet.

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VITAMIN E

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Due to greater interest in the anti-sterility properties of vitamin E, comparatively little attention has been given to its growth effect. Attempting the cure of male sterility, Evans and Burr ('27) noted that although cures were not effected, the administration of vitamin E was marked by an increase in the rate of growth. Subsequently, Evans ('28–'29) found that animals of both sexes reared on vitamin E-free diets showed, after the eighth month of life, but not before, a consistently poorer rate of growth than did rats receiving the same diet supplemented daily by a few drops of wheat germ oil. The same effect was observed with the unsaponifiable portion of this oil. Blumberg ('35) reported the use of a highly purified diet which was deficient in vitamin E. On this diet, normal early and middle growth failed to occur. Female rats showed subnormal growth in the first 5 weeks on the diet, and had plateaued in 18 weeks. In distribution and general properties, the growth principle resembled vitamin E. Some evidence obtained favored the view that wheat germ oil contained an unrecognized factor essential for optimal growth. Olcott and Mattill ('36) reported no improved growth in rats fed stock rations as compared to those on highly purified vitamin E deficient diets. The deficiency of vitamin E in the purified diet was established by proved sterility at 130 days. They conclude that the early growth effect of other observers could not be due to the vitamin E content of the supplements used.

Coward and co-workers ('29) and Guha ('31) produced a deficiency disease using special casein, but they did not prove that the curative principle was not vitamin E. Mason ('28–'29) believed that the beneficial effect of lettuce on growth was not due to its vitamin E content alone. The water-soluble early growth factor in liver, reported by Mapson ('32), would seem to be independent of any possible growth effect of vitamin E. Blumberg ('35) noted in the case of two male animals fed the experimental diet but with the addition of a concentrate (equivalent to 2 gm. per week of wheat germ oil—an amount in excess of the fertility requirement) that the growth curves were subnormal at the end of the twenty-eighth week, despite proved fertility by the mating test. This observation led to the investigation of the possible bipartite nature of the non-saponifiable matter of wheat germ oil with regard to the growth and anti-sterility effect attributed to it.

TECHNIC

The purified diet used was as follows:

Basal diet

	%
Casein (ether and alcohol extracted)	20
Yeast (ether extracted)	10
Salt mixture no. 185 (McCollum et al., '28)	4
Sucrose (commercial)	66

Supplements

Carotene—An ethyl laurate solution, fed at 2 drops per day equivalent to 0.08 mg. of carotene per rat per day. Solution prepared fresh every week and stored under carbon dioxide.

Irradiated ergosterol—An ethyl oleate solution of the resin, incorporated in the diet at 30 drops per kilogram, equivalent to 6 drops per kilogram of viosterol 250 D.

Ethyl linolate—A distilled oil, fed at 3 drops per day equivalent to 65 mg. per rat per day.

The details of preparation and feeding are as described by Blumberg ('35).

EXPERIMENTAL

A group of 58 rats, 26 males and 32 females, were placed on the basal diet with the supplements indicated. The animals were selected at weaning and weighed from 35 to 50 gm. each. These animals showed a slowing of growth at the tenth week and had plateaued at decidedly subnormal weights by the eighteenth week. The average plateauing weight of the females was 167 gm. (On stock diet females reach 190 to 210 gm.) which corroborates the observation of Blumberg ('35). For the males, the average weight when plateaued was 269 gm., whereas on stock diet males reach 350 to 450 gm. Each of the vitamin supplements was checked to assure optimal feeding levels by doubling the amounts and observing no additional response in animals plateaued on the diet as described. The possibility of excess dosage was checked by reducing the amount fed to one-half. Blumberg ('35) reported the completeness of this diet with respect to all known factors except vitamin E. When the sterility of the animals was proved by demonstrating non-motile sperm in the males and resorption of foetus in the females, supplements of variously prepared wheat germ oil concentrates were added.

A preparation of the unsaponifiable matter of wheat germ oil was prepared by hot saponification (2 hours at 80°C.) following the procedure of Burr and Burr ('30). The test animals, eight females and four males, showed weight gains averaging 27 gm. for the females and 26 gm. for the males in a period of 6 weeks on this supplement. The concentrate was fed at a level equivalent to 2.5 gm. of wheat germ oil per week. The females of each group were tested for fertility after they had been used to detect growth-promoting action of concentrates. The successful delivery of normal young when mated with a stock diet male was taken as evidence of the presence in the concentrate of ample vitamin E for protection against sterility. The growth response of these animals to the vitamin E concentrate of wheat germ oil confirms the observations of Blumberg ('35).

TABLE 1

Weight responses of rats to vitamin E concentrates; 6 weeks' feeding
(rats used were 18 weeks old and had previously plateaued on the basal diet)

SUPPLEMENT	ANTI-STERILITY POTENCY	WEIGHT GAIN IN 6 WEEKS IN GRAMS							
		Females				Males			
		Number	Average	Maximum	Minimum	Number	Average	Maximum	Minimum
Controls: vitamin E free basic diet	Negative	8	3	5	−1	2	−20	−26	−14
Ether soluble non-saponifiable matter of wheat germ oil = 2.5 gm. wheat germ oil per week	Positive	8	27	27	25	4	26	27	24
(1) Ether soluble non-saponifiable matter of cottonseed oil = 2.5 gm. of the oil per week	Positive	4	16	18	12	8	18	21	13
(2) Acetone soluble extract of (1) = 11.2 gm. cottonseed oil per week	Positive	4	12	16	6	0	..	..	..
(3) Petroleum ether soluble material of (2) = 11.2 gm. of cottonseed oil per week	Positive	2	5	6	4	2	8	10	6
(4) Methyl alcohol soluble material of (3) = 11.2 gm. of cottonseed oil per week	Positive	2	2	3	1	6	3	4	1
Non-saponifiable matter of wheat germ oil treated with ferric chloride in ether = 2.5 gm. of wheat germ oil per week	Negative	2	1.5	3	0	2	2	3	1
Non-saponifiable matter of wheat germ oil heated to 120 C. = 2.5 gm. of wheat germ oil per week	Positive	2	−1.5	1	−3	2	−11	−14	−8

With cottonseed oil as a starting point, numerous concentrates were made and fed to the test animal. The oil was subjected to a hot saponification (2 hours at 79°C.) and an ether extraction made, as in the case of the wheat germ oil. This extract, concentrate no. 1, when fed at an equivalent to 2.5 gm. of the original oil per week, showed both growth and anti-sterility effects. Subsequent purification of the vitamin was made as outlined by Olcott and Mattill ('31): The ether soluble material, concentrate no. 1 on table 1, was evaporated to dryness and dissolved in hot acetone. The acetone solution, concentrate no. 2, was cooled and filtered. The acetone was then evaporated and the residue extracted with petroleum ether. The petroleum ether solution, concentrate no. 3, was evaporated to dryness and extracted with methyl alcohol. The methyl alcohol soluble material was concentrate no. 4. The entire series of operations was carried out under carbon dioxide, using freshly distilled solvents. As can be seen from a consideration of table 1, the growth effect diminished as the purification of the vitamin proceeded, concentrate no. 4 showing practically no growth-stimulating potency and yet containing ample amount of vitamin E for the anti-sterility effect. The supplements, vitamin E concentrates, were administered with a small portion of the ration in the evening. By morning this had been consumed and ample diet for the day was placed in the feed cups. Concentrates nos. 2, 3 and 4 were fed at a level equivalent to 11.2 gm. of the original oil per week, this amount being divided equally into seven daily portions.

An attempt was made to destroy the vitamin E by treatment with ferric chloride (Waddell and Steenbock, '28) without altering the growth-promoting power of a wheat germ oil concentrate. The results showed a failure of the treated extract both in growth stimulation and in anti-sterility effect when fed at a level equal to 2.5 gm. of wheat germ oil per week.

The heating of the non-saponifiable matter of wheat germ oil to 120°C. for 1 hour in an autoclave, which should not

destroy vitamin E (Sure, '26), resulted in destruction of the factor in the concentrate which stimulated growth. The presence of sufficient vitamin E for anti-sterility effect was demonstrated in the two females treated with this preparation at a level equal to 2.5 gm. of wheat germ oil per week.

DISCUSSION

There are two possible explanations of the results found. First, a great difference exists between the amount of vitamin E required for anti-sterility effect and for growth; second, the concentrate formerly considered to contain one vitamin actually contains at least two—one growth stimulating and one having an anti-sterility effect. The evidence presented favors the possibility of a multiplicity of factors. Two preparations, the concentrate no. 4 and the autoclaved wheat germ oil concentrate, showed no growth effect and yet showed an anti-sterility potency in females of proved sterility. The preparation of crystalline α-tocopheryl allophanate, from which pure vitamin E was regenerated by Evans and coworkers ('36), should permit definite proof of this point when the preparation is available in sufficient amounts.

Suggestions have previously been made that vitamin E is in reality a complex. Grijns and Dingemanse ('33) obtained an extract of wheat germ which cured sterility in the male but not in the female. A second concentrate cured the female sterility and not the male. These authors used 40% alcohol in the presence of calcium hydroxide and kieselguhr for their separation. Blumberg ('35) suggested a possible growth and anti-sterility complex in wheat germ oil concentrates. Recently, Evans et al. ('36) by means of silver nitrate reduction tests, demonstrated that vitamin E activity is not the property of a single molecular species. This opens up the possibility that the two effects, growth and anti-sterility, may be due to slight alterations in a basic structure.

CONCLUSIONS

A report is made of an attempt to separate vitamin E concentrates into two fractions, one effective in curing sterility in rats and one effective in stimulating growth in these animals. The results show the presence of a factor stimulating growth, in concentrates formerly considered to exert only an anti-sterility effect.

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ADSORPTION OF VITAMIN B BY PLANT TISSUE (BY SOLANUM MELONGENA LINN. AND RAPHANUS SATIVUS VAR. LONGIPINNATUS BAILEY) WHEN PICKLED WITH SALT AND RICE BRAN [1]

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ONE FIGURE

(Received for publication December 2, 1936)

In a previous report (Miller and Abel, '33), it was shown that when a leafy vegetable, Chinese cabbage, Brassica chinensis, is pickled with a paste of salt and rice bran, the vitamin B (B_1) content of the cabbage is increased to almost four times the original value. The hypothesis was that the vitamin B from the rice bran either penetrated the tissues of the cabbage or was held by adsorption on the surface of the leaves. This paper gives the results of similar experiments with two other types of plant tissue: a fruit used as a vegetable, long eggplant, Solanum melongena Linn., and a root vegetable, daikon, Raphanus sativus var. longipinnatus Bailey.

The long slender eggplant, ranging from 4 to 10 inches in length and from 1 to 2 inches in diameter, is preferred by the Japanese to the larger round or oval type. It is one of the vegetables which these people pickle at home, alone or with other vegetables, using salt and such accessory substances as rice water, sake residue, kogi (fermented rice), miso (fermented soy bean and kogi), and rice bran.

[1] Published with the permission of the Director of the Hawaii Agricultural Experiment Station.

[2] Under the joint supervision of the University of Hawaii and the United States Department of Agriculture, Honolulu.

The long white radish or daikon is a much favored vegetable used in large quantities by the Japanese in Hawaii as in Japan. Pickled with salt and rice bran, it is called takuan,[3] and in this form or in the fresh state is utilized by the majority of Japanese families at every meal. Some takuan is prepared commercially in Hawaii, but most of it is imported from Japan. Small quantities are made at home by a few families.

METHODS

For experimental purposes, a uniform method of pickling the eggplant was followed. The eggplants, 6 to 10 inches in length and 1¼ to 1½ inches in diameter, were weighed. For each 100 gm. of vegetable, 119 gm. of rice bran, 21 gm. of table salt and 210 gm. of water were made into a paste. Only firm whole eggplants with unbroken skin and with stem and calyx in place were used. The fruits were rolled gently with the hand on a smooth surface to soften them slightly. They were then placed in alternate layers in a wooden shoyu tub with the paste of salt and rice bran. A wooden cover fitting loosely into the tub was placed on the contents and weighted with a stone. The eggplant and paste were mixed each day and left for 72 hours. The eggplants, washed free of bran and salt, were placed in a covered glass jar in an electric refrigerator until needed. To secure a uniform sample each day, a whole eggplant was chopped fine and the quantities fed to rats were weighed to within $\frac{1}{100}$ gm. A fresh lot of eggplant was bran-salt-pickeled twice a week.

The product designated as takuan A was prepared in the nutrition laboratory as follows: Large daikon 12 to 15 inches long and 1¼ to 2 inches in diameter were washed gently so as not to break the skin, then cut crosswise into two approximately equal pieces. After the daikon were weighed, salt equal to 5% of their weight was sprinkled over the daikon placed in layers in a clean shoyu tub. A fitted board was placed on the daikon and weighted with a heavy stone. The

[3] Takuan has been eaten in Japan for 300 years or more. The term Takuan (sometimes spelled takuwan) is usually associated with a priest of that name, who, if he did not originate the method of pickling, did much to popularize its use.

daikon were mixed once a day and were removed after 72 hours from the resulting brine. This brine was discarded and the pre-salted daikon weighed without washing. Salt equal to 4%, and rice bran equal to 50%, of the pre-salted weight of the daikon were mixed and placed in alternate layers with the daikon in the clean shoyu tub and weighted with wooden cover and stone as before. The daikon and bran paste were mixed once daily and allowed to pickle for 1 week. The pickled product, takuan, was taken out of the tub, washed thoroughly and stored in glass jars in an electric refrigerator. Because of the large size of each root, it was impossible to use a whole one each day for feeding purposes. Cross sections from the center of half a root were cut in small pie-shaped pieces and weighed to within $\frac{1}{100}$ gm. A fresh lot of takuan was prepared each week.

Takuan B, used for the experiments, was commercial takuan imported from Japan.

The feeding methods were those previously described (Miller and Abel, '33) with the additional use of the international standard vitamin B preparation for positive control rats. Daily feeding means every day except Sunday.

The pH values of the extracted plant juices, made by grinding the tissues and squeezing them in cheese cloth, were determined as previously reported (Miller and Abel, '33).

RESULTS

Eggplant. Forty-five rats given a vitamin B-free diet were used for the experiments and fed as listed in table 1. The results, summarized in figure 1 and table 1, show that rats receiving 2 gm. of fresh eggplant daily gained less than the rats fed the standard and that rats fed bran-salt-pickled eggplant equivalent to 2 gm. of fresh eggplant gained as much as those fed 3 gm. of the fresh vegetable.

The loss in weight resulting from pickling twenty-two samples of eggplant in salt-rice-bran-paste varied from 10 to 32% with an average of 17%. The quantity of bran-salt-pickled eggplant equivalent to 2 gm. of the fresh vegetable was calculated for each lot according to the loss in weight.

Samples of one eggplant from each of twelve lots of the vegetable purchased over a period of 4 months varied slightly in pH, from 5.1 to 5.7 with an average of pH 5.4. Twenty-two lots of bran-salt-pickled eggplant made from the twelve lots of fresh eggplant all showed a definite increase in acidity, ranging from pH 5.1 to 4.5 with an average of pH 4.8.

TABLE 1

Results of feeding fresh and bran-salt-pickled vegetables as the sole source of vitamin B

SOURCE OF VITAMIN B	NUMBER OF RATS	WEIGHT OF SUPPLEMENT FED DAILY	AVERAGE WEIGHT OF RATS			AVERAGE GAIN IN 6 WEEKS	APPROXIMATE UNITS PER 100 GM.
			Initial	When started on supplement	Final		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
Negative control	10	0.0	41	73[1]	53		
Standard	10	0.005	39	65	102	37	
Eggplant, fresh	6	2.0	38	69	94	25	20
Eggplant, fresh	7	3.0	42	74	130	56	
Eggplant, bran-salt-pickled	12	1.6–1.7[2]	40	72	131	59	40
Negative control	11	0.0	37	69[1]	54		
Standard	12	0.005	37	67	98	31	
Daikon, fresh	10	8.0	39	67	106	39	6
Takuan A (nutr. lab.)	11	0.5	38	65	113	48	110–120
Takuan B (commercial)	10	1.0	39	71	97	26	40

[1] Weight at the end of the depletion period.

[2] Equivalent to 2 gm. of fresh.

Daikon and takuan. Fifty-four rats given a vitamin B-free diet were used to test the vitamin B content of daikon and takuan. The results are summarized in table 1 and figure 1.

That fresh daikon contains little vitamin B is demonstrated by the fact that it was necessary to feed 8 gm. daily in order to secure a gain comparable to that of rats fed daily 0.005 gm. of the vitamin B standard.

Table 1 also shows that the fresh pickled daikon (takuan A) prepared in our laboratory had a greater vitamin B value than

the commercial product, 0.5 gm. of the freshly prepared takuan (takuan A) producing about double the gain in weight resulting from 1.0 gm. of the commercial takuan (takuan B). It is quite possible that the freshly made commercial preparation contains as much vitamin B as our laboratory preparation, but gradually decreases in vitamin B through standing.

Daikon loses much water as a result of the pickling process, the percentage loss being about the same after the pre-salting

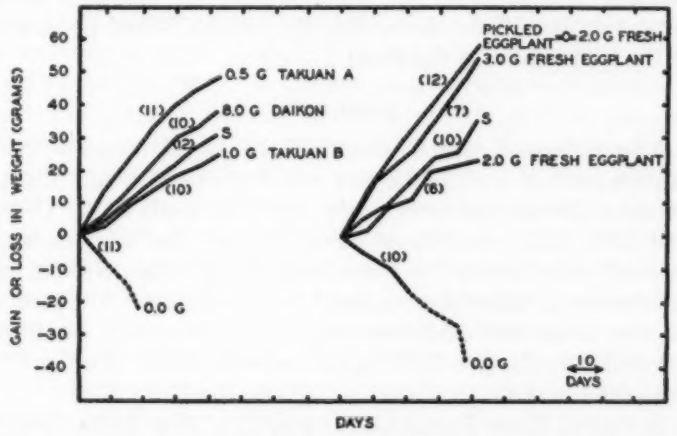

Fig. 1 Average gain in weight of rats fed fresh and bran-salt-pickled vegetables and the vitamin B standard. S = 0.005 gm. of the International Standard. The broken line indicates the occurrence of the first death in the control group. The numbers in parentheses show the number of rats used for each test.

as after the final pickling with salt and rice bran. The average loss in weight for nine samples was 64%, the minimum 62%, the maximum 74%. The weight of takuan equivalent to the fresh daikon was not calculated for each lot of takuan A prepared but 0.5 gm. was fed throughout the experiment.

The average pH of four samples of fresh daikon was 5.8, with little variation between the samples. As a result of the 3 days' pre-salting, there was practically no change in acidity, the average pH being 5.7. After a week in salt-rice-bran-paste, the average acidity increased to pH 4.8.

Limitations of space do not permit the inclusion of the results of preliminary experiments on twenty-five rats of which some were fed fresh daikon, some commercial takuan, and some used as negative controls. Eight of the group were matched litter mates fed equal portions of the outside and the inside of the takuan. When that portion representing one-third of the diameter of a cross section was taken from the center of the takuan root and fed to rats as the sole source of vitamin B, it induced just as great growth response as the outer portion. This shows that the vitamn B had penetrated to the very center of the root.

DISCUSSION

The extracted plant juices of the eggplant and of the takuan reached a pH of 4.8 as a result of the bran-salt-pickling process, the cabbage previously reported, a pH of 4.7 (Miller and Abel, '33). Acidity of about this pH has been shown to be most satisfactory for the adsorption of vitamin B on such substances as fuller's earth and Lloyd's reagent. The medium of rice bran, salt and moisture seems to create conditions favorable to the penetration and adsorption of vitamin B by any plant tissue.

A report from Japan (Kato and Ho, '30) states that the rice-bran paste from pickling vegetables contains many organisms of which a lactic acid bacillus is the most abundant. As in fermented cabbage (sauerkraut) and salt-cured cucumbers, lactic acid is the principal acid produced.

Dutcher ('32) and others have shown that certain micro-organisms possess the property of synthesizing the vitamin B complex. No study of the bacteria and yeasts involved in the bran-salt-pickling process has been made in the nutrition laboratory. However, since rice bran is extremely rich in vitamin B, a water soluble substance, it seems reasonable to assume that the vitamin B of the bran-salt-pickled products is derived from the original vitamin B of the bran; whether the quantity of vitamin B may or may not be augmented by micro-organisms present is a matter of little importance under these conditions.

Bran-salt-pickled eggplant contains per unit of weight only about twice the quantity of vitamin B as the fresh (table 1). Considering the loss in weight due to bran-salt-pickling, the increase in vitamin B content is only about 1½ times the original value.

On the basis of units of vitamin B per 100 gm., takuan has about eighteen times as much vitamin B as the fresh daikon from which it is made (table 1). However, so much moisture is lost in the process of pickling that for the equivalent weight of fresh daikon, takuan contains only about seven times as much vitamin B as the original daikon.

Even though there is always considerable loss of water from the plant tissues as a result of the pickling process, nevertheless vitamin B, being water soluble, penetrates to the innermost tissues of the vegetables and is thus not removed by thorough, though not prolonged, washing in tap water. Our experiments show that, as a result of the bran-salt-pickling process, the vitamin B content of a leafy vegetable may be increased four times, of a fruit one and one-half times and of a root seven times its original value.

Due to its thick cuticle, the eggplant has a relatively impervious epidermis, a surface through which the vitamin B and salt can penetrate only slowly when the skin is unbroken and there are no cut ends. The daikon on the other hand has a more permeable epidermis and relatively less cuticle. Further, the ends were cut so that the soluble materials might easily penetrate the fibro-vascular bundles and, having once reached the innermost tissues, would not readily be lost.

In contrast with pickling in salt alone (Miller and Abel, '33), which decreases the original quantity of vitamin B, the general use of rice bran for pickling vegetables as a method of preservation in the orient and elsewhere is a practice much to be commended because it utilizes a product of high nutritive value (rice bran) otherwise little used for human consumption and because it results in an increased vitamin B intake where that vitamin is likely to be low.

SUMMARY

1. As a result of pickling long eggplant (Solanum melongena Linn.) in a paste of salt and rice bran for 3 days, the original vitamin B content of the eggplant is increased about one and one-half times.

2. Takuan, a product prepared from daikon (Raphanus sativus var. longipinnatus Bailey) by pickling in a paste of salt and rice bran for a week, has about seven times as much vitamin B as the original. Commercial takuan that had been pickled for some months had a vitamin B value less than half of that prepared in the laboratory.

3. As a result of pickling in a paste of salt and rice bran, plant tissues—whether leaf, fruit or root—reach a pH of 4.7 or 4.8, a condition that seems to be conducive to the adsorption and retention of vitamin B from the rice bran.

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SUP

SUPPLEMENT

PROCEEDINGS OF THE FOURTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

MINUTES OF MEETING OF AMERICAN INSTITUTE OF NUTRITION, MEMPHIS, TENN., APRIL 21, 1937

The fourth annual meeting of the American Institute of Nutrition was held in Memphis, Tenn., at the Hotel Peabody on April 21, 1937. Although only 90 members and 228 guests registered there were between 400 and 500 in attendance during the entire program.

President Eugene F. DuBois presided at the scientific sessions and business meeting. The scientific program was initiated promptly at 9.30 A.M. and with but few exceptions the speakers were thoughtful in consuming only the time allotted for the presentation of their papers. This helpful cooperation on the part of all the speakers enabled the entire program to move according to schedule and therefore with ease, satisfaction and enjoyment. All the papers listed on the program were given.

The business meeting convened at 11.30 A.M. and was called to order by Pres. Eugene F. DuBois.

The members stood in silence in recognition of the deaths of W. McKim Marriott and Winfred W. Braman.

The report of the nominating committee was presented by Arthur H. Smith, chairman. It was moved by Harold Goss and seconded by Paul E. Howe that the secretary cast a unanimous ballot for the slate as presented. The motion carried and the following officers were elected:

	Associate editors
President, Mary S. Rose	W. H. Chambers
Vice-president, E. V. McCollum	A. G. Hogan
Secretary, I. G. Macy	J. B. Brown
Treasurer, G. R. Cowgill	P. A. Shaffer
Councillor, P. E. Howe	J. L. Gamble

The report of the treasurer was read by George R. Cowgill. L. A. Maynard and C. A. Elvehjem, auditors reported the books correct. It was moved by Paul E. Howe and seconded by E. M. Nelson that the treasurer's report be accepted. The motion carried. The council recommends that the annual dues remain $1.00 during the coming year.

President DuBois appointed the following persons as members of the nominating committee for 1938: R. M. Bethke, chairman; A. H. Smith, W. M. Boothby, H. B. Lewis and Agnes F. Morgan.

The report of the committee on vitamin nomenclature was heard. E. M. Nelson, chairman of a committee composed of himself, E. V. McCollum and H. C. Sherman, after discussing the activities of the committee moved that the recommendations as submitted by the committee be approved. The motion was seconded by V. C. Myers and approved by the members. Although a large majority of the members favored dropping the final 'e' in the spelling of thiamine the final decision was left to the discretion of the committee. The committee was requested to continue its work.

The recommended changes in the By-Laws as submitted to the membership preceding the meeting in accordance with the constitution were discussed by President DuBois. He remarked that the council interprets Article VII to mean that the editor can be reappointed as stated in the by-laws. It was moved by R. M. Bethke and seconded by C. A. Elvehjem that the proposed changes be adopted. The motion carried.

President DuBois discussed a request from the Children's Bureau for qualifications of nutritionists in public health departments. In accordance therewith he appointed the following committee to study the request and make recommendations back to the council for their consideration: Mary S. Rose, chairman; R. M. Bethke, L. J. Roberts, W. C. Rose and Icie G. Macy.

The council recommended that a questionnaire be prepared and sent to the members to ascertain their wishes on the type of program desired and the place and time of meeting. A

committee composed of George R. Cowgill, R. M. Bethke and Icie G. Macy was appointed to draw up the questionnaire. President DuBois stressed the importance of suggestions from the members in guiding the council regarding nature of programs, etc. The council expressed the desire that the members of the institute themselves present the papers at the meeting since in their judgment it seems prudent at present at least to emphasize mature contributions rather than using the program for a training ground.

President DuBois announced that the council had charged R. M. Bethke and George R. Cowgill with the responsibility of submitting a resolution on the food, drug and cosmetic bill. The following statement was heard and approved by the members:

Resolved that the American Institute of Nutrition favors the passage of federal legislation which will control more effectively than at present the quality of and representations for food, drug and cosmetic products.

Mary S. Rose submitted the following resolution in connection with the work of the health organization of the League of Nations:

The American Institute of Nutrition endorses the report on Physiological Bases of Nutrition prepared by the Nutrition Committee of the Health Organization of the League of Nations as a guide for various countries for procuring optimum nutrition for their populations.

The recommendations regarding energy and protein requirements are in general harmony with standards approved by the members of the American Institute of Nutrition and the emphasis on liberal amounts of protective foods as sources of minerals and vitamins especialy for expectant and nursing mothers and for children of all ages is of the greatest significance for the welfare of the oncoming generation.

The report is commendable for calling attention to the importance of milk as a protective food and for setting standards in terms of common protective foods to which may be added such sources of energy as local conditions make practicable.

T. M. Carpenter moved that the resolution be adopted by the American Institute of Nutrition and sent to the medical director of the Health Organization of the League of Nations.

The council recommended that the 1938 meeting be held prior to the federation meeting.

It was moved by Paul E. Howe and seconded by E. M. Nelson that the secretary cast a ballot for the nine applicants recommended by the council for membership in the American Institute of Nutrition. The list follows:

H. A. Almquist	P. C. Jeans	G. Stearns
H. M. Dyer	J. M. Orten	J. Tilt
C. F. Huffman	W. H. Sebrell	A. White

John R. Murlin, managing editor, reported on The Journal of Nutrition. He stated that it would not be possible to increase from two to three volumes per year on account of finances. He expressed the hope that this might be possible in another year.

The council extended its thanks to the local committee and to the Hotel Peabody management for the splendid arrangements provided for the meeting and for the many courtesies extended to the members and their guests.

The meeting was adjourned.

Respectfully submitted,

ICIE G. MACY, *Secretary.*

CONSTITUTION

1. The name of the proposed society is the 'American Institute of Nutrition.'

2. The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest.

3. The management of the American Institute of Nutrition shall be vested in a council consisting of the President, Vice-President, Secretary, Treasurer and three additional members.

BY-LAWS [1]

Article I—*Membership*

Section 1. There shall be two classes of members, members and emeritus members. The number of members shall be limited to 250; exclusive of emeritus members.

Section 2. *Elegibility for membership*: Members.. Qualified investigators who have independently conducted and published meritorius original investigations in some phase of the chemistry or physiology of nutrition and who have shown a professional interest in nutrition for at least 5 years shall be eligible for membership in the Society. Emeritus Members. Members in good standing who have reached the age of 65 years shall become emeritus members. A member in good standing and for sufficient reason may by vote of the Council be made an emeritus member. Emeritus members shall be entitled to vote but not hold office.

Section 3. *Nomination*: Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting.

Section 4. *Election to membership*: A. A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility. B. A majority of the ballots cast shall elect.

Section 5. *Forfeiture*: If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least 2 weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion; and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

Article II—*Meetings and Quorum*

Section 1. *Annual*: The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation.

Section 2. *Special*: A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifteen members of the Society. Notice specifying the purpose of such meeting shall be mailed to each member at least 10 days previous thereto. The Council shall select the places at which meetings shall be held.

Section 3. *Quorum*: Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date.

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[1] All amendments to April 21, 1937 are incorporated.

Article III—*Officials*

Section 1. *Officers*: The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society. Their terms of office shall commence with the close of the annual meeting at which they are elected.

Section 2. *Council*: The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and shall be known as 'The Council.' (When this provision is first put into effect one member shall be elected for 1 year, one for 2 years and the third for 3 years.)

Section 3. *Duties of Officers*: The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions.

Article IV—*The Council*

Section 1. *Powers*: The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York. A provisional charter was issued to the American Institute of Nutrition under date of September 27, 1928.

Section 2. *Reports*: The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws.

Article V—*Nominating Committee*

Section 1. *Membership*: A. The Nominating Committee shall consist of five members appointed for the coming year by the retiring President. Members who have served on the Nominating Committee for 2 consecutive years shall be ineligible for reappointment until after a lapse of 1 year. B. The President shall designate one member to be Chairman of the Nominating Committee.

Section 2. *Nomination of Officials*: A. The Nominating Committee shall make at least one nomination for each of the four offices, for each of the additional positions on the Council to be filled by vote of the members and for each of the positions on the Editorial Board to be vacated at the time of the annual meeting. Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee. B. The nominations by the Nominating Committee shall be transmitted to the Secretary at least 6 weeks before the annual meeting at which they are to be considered. C. The Secretary shall send to every member, at least 2 weeks before the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail, returning to the Secretary the marked ballot in the envelope provided, at such a time and place as the Secretary may designate, or the ballot may be delivered to the Secretary at the beginning of the business session at which the elections are to

take place. D. Additional nominations for the officers and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting.

Section 3. *Election of Officials*: A. At the beginning of the business session the Secretary shall present to the tellers, appointed by the President, the ballots submitted by the members and the ballots shall be counted forthwith. B. A majority of votes cast shall be necessary to elect an official.

Section 4. *Filling of Vacancies*: A. The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society. B. The President of the Society shall fill all vacancies in appointive positions.

Article VI—*Financial*

Section 1. *Dues*: The dues shall be the annual cost of subscription to The Journal of Nutrition for members plus an annual assessment which shall be determined by majority vote at the annual meetings, upon recommendation of the Council, and shall be due within a month after the annual meeting. Emeritus members are not required to pay assessments nor to subscribe to The Journal of Nutrition.

Section 2. *Expenditures*: No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council.

Section 3. *Penalty for non-payment of dues*: A. Members in arrears for dues for 2 consecutive years shall forfeit their membership. B. Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated.

Article VII—*The Journal of Nutrition*

Section 1. The American Institute of Nutrition designates The Journal of Nutrition as its official organ of publication.

Section 2. In accordance with the expressed wish of The Wistar Institute of Anatomy and Biology, owner and publisher of The Journal of Nutrition, the American Institute of Nutrition shall nominate members of the Editorial Board for its official organ. A. The editorial management of The Journal of Nutrition shall be vested in an Editorial Board consisting of an Editor and twelve Associate Editors. B. The Editor shall be chosen by the Editorial Board to serve a term of 5 years. The Board shall also have the power to designate annually one or more of its members to serve as Assistant to the Editor. C. Three members of the Institute shall be nominated by the Nominating Committee for membership on the Editorial Board each year to serve a term of 4 years, replacing three retiring members. In the event of a vacancy in the membership of the Editorial Board occurring through death or other reason, the Nominating Committee, for each such vacancy to be filled shall make an additional nomination. In this event the nominees elected who receive the greatest number of votes shall serve the longest term of vacancies to be filled. D. Retiring members of the Editorial Board shall not be eligible for renomination until 1 year after their retirement.

Article VIII—*Papers on Scientific Subjects*

Section 1. The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings.

Article IX—*Changes in Constitution and By-Laws*

Section 1. Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least 1 month before the date of the meeting at which they are to be considered, and must be signed by at least three members. The Secretary shall send a printed copy of any proposed change to each member at least 2 weeks before the next meeting and shall notify all members that they may vote by proxy.

Section 2. If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made.

ABSTRACTS OF PAPERS READ

Organic vs. inorganic compounds as sources of mineral elements in nutrition. Genevieve Stearns (by invitation), Department of Pediatrics, State University of Iowa.

The retentions of sodium, potassium, calcium, phosphorus and iron have been studied when these elements were given to infants in foods, and as inorganic salts. In general either source was well utilized by the infant.

The quantity of intake of either of the monovalent ions studied was apparently without significant effect on the retention of any of the elements studied. The polyvalent ions, however, showed a greater degree of interrelationship. An oxalate containing food given to infants decreased the retentions of both calcium and iron.

The quantity of an element ingested, both in absolute amount, and in relation to the other minerals fed, seems to be of more importance for infant feeding than whether the element is fed as an organic or inorganic salt.

The influence of sex on utilization of iron. Helen J. Hubbell (by invitation) and Mary S. Rose, Nutrition Laboratory, Teachers College, Columbia University.

Rats made anemic by milk feeding were fed milk supplemented by iron and copper in amounts proportional to body weights until blood hemoglobin reached 14 gm. per 100 cc., when their bodies were analyzed for iron. Three levels of iron intake were studied.

A series fed 0.0019 mg. per gram of rat daily for a period averaging 45 days for seven males and 49 days for eight females, averaged for hemoglobin 14.3 gm. and 13.8 gm., respectively; for body iron 030.0 $\pm$ 0.0003 mg. per gram of rat for males and 0.038 $\pm$ 0.0007 mg. for females.

A series fed 0.038 mg. per gram of rat daily for a period averaging 39 days for eight males and 38 days for eight females, averaged for hemoglobin 14.1 gm. and 14.4 gm., respectively; for body iron 0.034 $\pm$ 0.0006 mg. per gram of rat for males and 0.038 $\pm$ 0.0007 mg. for females.

A series fed 0.0057 mg. of iron per gram of rat gave no evidence of further storage of iron but similar sex differences in iron content of bodies.

In each series the females showed a higher iron content by 13, 12 and 15%, respectively. No sex difference was found in the iron content of anemic controls analyzed at the end of the depletion period.

The nutritional status of children as judged by the creatinine-height coefficients. Amy L. Daniels, Iowa Child Welfare Research Station, State University of Iowa.

The study is concerned with the creatinine-height coefficients of pre-school children from two socio-economic groups. On the assumption that the creatinine-height is a measure of the nutritional status, these have been compared with the norms of weight for height, the predicted weights based on body build, and variations from the normal Pelidisi. In certain instances, the skeletal age has been considered in relation to these nutritional indices.

The calcium retentions of pre-school girls at different levels of calcium intake. Julia Outhouse, Gladys Kinsman (by invitation), Dorothy Sheldon (by invitation), Irene Twomey (by invitation), Janice Smith (by invitation) and Milicent Hathaway (by invitation), Department of Home Economics, University of Illinois, Urbana, and H. H. Mitchell, Department of Animal Husbandry, University of Illinois.

Five pre-school children were fed different levels of calcium in conjunction with a diet ample in essential nutrients. Excreta and food were collected and analyzed in weekly periods. During the first 15 weeks, generous amounts of di-calcium phosphate were fed, in order that saturation of the bones might be obtained. This procedure, it was assumed, would tend to erase differences in storage due to differences in the previous regimens of the children. During this period the retentions varied considerably from week to week and from child to child. Three children retained more calcium during the first 5 weeks than during the last 6 weeks. These results were reversed in the other children. At the end of 15 weeks, the quantity of calcium given was decreased to 350 mg. daily. This represented from two to seven times the amount of the average retention on the previous high level. Retentions were reduced in all but one child. At the end of the eighth week the calcium content was increased to 600 mg. daily through the addition of milk solids equivalent to 200 cc. milk. The increased retentions were proof that none of the children was receiving enough calcium on the preceding period of low calcium intake.

Effect of a milk supplement on bone development in institution children as indicated by ossification of bones of the wrist. Lydia J. Roberts and Vera MacNair (by invitation), Department of Home Economics, The University of Chicago.

The effect of adding a pint of milk, both irradiated and non-irradiated to the diets of institution children was studied over the period of a year. Roentgenograms of the wrist were taken at the beginning and end and the progress of bone development, as indicated by Carter ossification ratio, followed. Comparisons were made for twenty-four matched trios consisting of one child each from control, plain milk and irradiated milk groups.

All groups at the outset were retarded in respect to the Carter norms and were still retarded at the end of the study, but the supplemented groups reduced their deficits to a greater degree than did the control.

1. The percentage of children in more favorable relation to the standard at the end was 17 for the control, 50 for the milk, and 46 for the D-milk group.

2. The mean gain in Carter points was 6.0 for the control, 8.3 for milk and 8.2 for the D-milk group, an advantage of 2.3 and 2.2 points, approximately a third of a year's quota in favor of the supplemented groups.

3. The control group gained 79% of its expected quota; the supplemented groups each 115%.

4. In the twenty-four matched trios the control child made the greatest annual gain in 21%, the milk child in 44%, and the D-milk child in 35% of the comparisons.

Metabolism studies in human beings on the age disposition to ketosis. Walter Heymann (introduced by V. C. Myers), Babies and Children's Hospital, and the Department of Pediatrics, Western Reserve University.

Thirty-six persons of varying age (2 months to 35 years) were placed for 3 consecutive days on a ketogenic diet with a ratio of 2.5: 1. Development of ketosis was detected daily by quantitative determination of acetone excretion in the urine. It was found that up to the age of 6 months ketonuria remains minimal. The ability to develop ketonuria begins at the age of 7 to 8 months, increases continuously during the first 3 years, reaches a peak at 4 to 8 years and decreases during prepuberty where it equals values obtained in adults, values which are still much higher than those found in young infants.

Differences in the production as well as in the utilization or excretion of ketone bodies may account for these results. It was found, however, that infants and children began to excrete acetone as well as sodium acetoacetate at the same level of dosage in a quantitatively comparable manner, when they were fed these compounds. Consequently, it must be concluded that the age disposition to ketosis is due to a difference in ability to produce acetone bodies.

Salt metabolism in muscular activity. D. B. Dill, Jost Michelsen (by invitation) and H. T. Edwards (by invitation), Fatigue Laboratory, Morgan Hall, Harvard University.

The need for increased intake of sodium chloride in periods of excessive sweat secretion is becoming generally recognized. The diets of athletes, of laborers in heavy industries, and of patients with fever should provide for extra-renal salt loss.

It is not generally known that salt concentration in sweat depends on acclimatization, environmental factors and personal idiosyncracy. If work is undertaken in midwinter in an arid environment at 40 to 45°C., the concentration of salt in sweat may vary from 45 to 89 millimolar in different individuals. In one subject the concentrations were 87, 89 and 89, respectively, in three periods of work totaling 4½ hours. The water excretion was about 4 liters; only one-tenth as much was ingested and thirst was satisfied. Serum chloride decreased 4%, osmotic pressure increased 7%, and serum protein increased one-fourth. The urine became chloride-free, and heat cramps were experienced.

The same subject after acclimatization at Boulder City in the summer of 1932 walked for 7.1 hours in the desert with an air temperature of about 40°C. He excreted about 9 liters of water and drank two-thirds as much. Serum chloride remained constant and serum protein increased one-fifth. The indirect measurements at Boulder City indicated that the salt concentration in sweat was about one-third that found on the same subject in Boston without acclimatization to high temperature.

The influence of inorganic salts in the diet on the changes in electrolytes and water of the tissues of the rat. Ercel S. Eppright (by invitation) and Arthur H. Smith, Laboratory of Physiological Chemistry, Yale University.

Young, rapidly growing rats were fed a basal low-salt ration plus various mineral supplements in quantities normally consumed by rats eating ad libitum an adequate synthetic diet. Energy, protein, and vitamin intakes were restricted to the average consumption of rats on low-salt diet.

Analysis of tissues of animals sacrificed after 60 days showed that the dietary inorganic matter profoundly influences tissue hydration. Sodium chloride unsupplemented by other salts results in excessive hydration, general except for skeletal muscle. With potassium added to sodium chloride, the tendency toward excessive hydration is slightly reduced. When calcium is the predominant basic ion, dehydration prevails in many tissues.

With Osborne-Mendel salt mixture in the diet, there is close agreement between extracellular volumes of water as calculated from chloride or from sodium content of muscle and serum. Lacking all the salts or calcium and phosphorus, there is an excess of sodium in muscle unaccounted for by that in extracellular water as calculated from chlorides.

Potassium and sodium are mutually antagonistic to their retention in muscle; diminished potassium and augmented sodium accompany poor nutritional state. The ratio of potassium to sodium in muscle, much distorted on sodium chloride or sodium chloride plus potassium regimes, is normal when calcium is ingested.

The significance of dietary calcium in motivating shifts of electrolytes and water in tissues is emphasized by this study.

Vitamin B fractions. Their nomenclature and functions. C. A. Elvehjem, Department of Agricultural Chemistry, University of Wisconsin.

The designation and the function of six of the factors which have been included in the so-called B complex will be discussed according to the following outline:

1. Vitamin B or B_1. Antineuritic vitamin. Functions in carbohydrate metabolism, especially pyruvic acid metabolism. There is no evidence that it has any direct relationship to nerve degeneration.

2. Flavin. Lactoflavin. Designated vitamin G or B_2 by Kuhn, Karrer, Booher, Lepkovsky and Jukes, and others. Indispensability demonstrated for rats and chicks. Undoubtedly functions in human metabolism. Is the prosthetic group in flavo-proteins.

3. Antipellagric factor. Designated vitamin B_2(G) by Elvehjem and Koehn human P-P factor or canine blacktongue factor by Birch, György, and Harris; 'filtrate factor' or 'factor 2' by Lepkovsky and Jukes. Cures pellagra-like symptoms in chicks and blacktongue in dogs. A concentrate of this factor has proved active in human pellagra.

4. Rat antidermatitis factor. Designated vitamin B_6 by György, by Chick and by Harris; 'Factor 1' by Lepkovsky and Jukes; and vitamin B by Hogan and Richardson. There is some variation in the reports on the stability of vitamin H and vitamin B_6. Function and relation to human nutrition not definite.

5. Antiparalytic factor. Designated vitamin B_4 by Reader and by Keenan et al. Necessary for normal brain structure. No work on relation to humans.

6. Factor W. Designated alcohol-ether precipitate factor by Elvehjem, Koehn and Oleson. Its function may be related to flavins and tissue respiration.

The tripartite nature of vitamin G. Samuel Lepkovsky and Myrtise E. Krause (by invitation), Poultry Division, University of California, Berkeley.

Vitamin G is now known to consist of three separate vitamins, one, lactoflavin, obtainable in crystalline form, and the other two available only as extracts. The latter two can be differentiated from each other biologically, one (factor 1)[1] curing a 'specific' dermatitis in rats. The other (factor 2) also cures a dermatitis in chicks. In rats it cures a disorder characterized by loss of hair sometimes accompanied by dermatitis, and a swelling of the eyelids of such severity they frequently stick together. Both are growth promoting in both rats and chicks. Factor 1 can be separated chemically from factor 2 by its adsorption on fullers' earth, and by its greater heat stability in alkaline medium.

An interrelationship apparently exists between factor 1 and both lactoflavin and factor 2. Rats maintained on minimal amounts of factor 1, will grow and remain free of dermatitis provided both lactoflavin and factor 2 are present in adequate amounts. They will, however, develop the 'specific' dermatitis and their growth will be disturbed if either lactoflavin or factor 2 is withheld or fed in inadequate amounts. Similar results were obtained by Birch and György[2] when linoleic acid was withheld. It is noteworthy that all three factors so physiologically interrelated with factor 1, will themselves cure some sort of skin disturbance.

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[1] Factor 1 is probably identical with vitamin B_6, the difference being that only vitamin B_1 and lactoflavin are fed for testing vitamin B_6, whereas vitamin B_1, lactoflavin, and factor 2 must be fed for factor 1 tests.

[2] Birch, T. W. and P. György 1935 Biol. Chem. J., vol. 30, p. 304.

Vitamin B_2 deficiencies as influenced by dietary carbohydrates. Agnes Fay Morgan, Bessie B. Cook (by invitation) and Helen G Davison (by invitation), Laboratory of Household Science, University of California.

Lactose, sucrose and cornstarch were used as 54 to 70% of the basal diets fed to young rats deprived of one or more of the vitamin B_2 factors. Growth, dermatitis, cataract formation, blood and urine sugar and calcium were determined on animals kept in individual screen-floored cages on these regimes for 8 to 16 weeks.

Three factors of the vitamin B_2 complex were used, a wheat germ preparation of vitamin B_6, a rice bran preparation of the filtrate factor and crystalline lactoflavin. Crystalline vitamin B_1 (Merck) was given in ample amount to all the rats. The three vitamin B_2 preparations were given each alone, all combinations of two together as well as all three together.

The lactose diet with no vitamin B_2 factors produced as good conditions as with B_6 and flavin. The filtrate factor, however, improved growth. The cornstarch diet was but little improved by addition of filtrate factor but much improved by both B_6 and flavin. The sucrose diet gave better growth than the others when supplemented by all the factors but apparently supplied none of them spontaneously.

Thus the intestinal flora supported by lactose produces flavin and B_6, cornstarch carries the filtrate factor or favors its intestinal formation, and sucrose neither carries nor allows the intestinal production of any of these factors.

The vitamin B requirement of children. The effect of varied ingestion of vitamin B upon the food consumption of children. Frederic W. Schultz and Elizabeth M. Knott (by invitation), Department of Pediatrics, The University of Chicago.

Quantitative studies over an extended period of time have been made of the total daily weights of food consumed by two groups of children. Group I included thirty-six ambulatory children, 5 to 10 years of age, living at the Country Home for Convalescent Crippled Children, which is affiliated with The University of Chicago. Group II included twenty-two girls between the ages of 9 and 11, whose health was excellent, and who had been living for some time under the optimum conditions afforded by the Mooseheart Home for Child Training. Following control periods, the vitamin B level of the diets, both for the crippled children and the normal children, was augmented first through use of a stabilized wheat germ product and second by means of crystalline vitamin B. When results were calculated on the basis of grams of food consumed per square meter of body surface, the increased intake of vitamin B increased the food consumption from 12.5% to 28.6%. The quantity of vitamin B in the diet which produced the highest food consumption was approximately 22 International standard units per kilogram of body weight for the younger children, and about 19 units per kilogram for the older children. This quantity of vitamin B is comparable to the amounts (reported in J. Nutrition, vol. 12 p. 597, 1936) which produced the highest retention of the vitamin. Twenty units of vitamin B per kilogram may therefore tentatively be considered as the optimum vitamin B requirement of children.

Panmyelophthisis with hemorrhagic manifestations in rats on a nutritional basis. Paul György (introduced by V. C. Myers), Babies and Children's Hospital and Department of Pediatrics, School of Medicine, Western Reserve University.

Rats kept on a vitamin B complex deficient diet supplemented with vitamin B_1 plus synthetic lactoflavin developed the characteristic picture of 'acrodynia' in 6 to 15 weeks. Treatment with potent purified B_6 concentrates, prepared from wheat germ or molasses, resulted in improvement of the specific dermatitis. This improvement, however, was followed in many cases not by complete recovery but

by severe anemia, together with symptoms of a hemorrhagic diathesis, such as blood effusions in the skin, epistaxis, melena, hematuria, and, frequently, bilateral adrenal hemorrhage. In all these cases the rat 'acrodynia' was in remission before the hemorrhagic diathesis and anemia became manifest. In several rats, spontaneous improvement of the specific 'acrodynia' was noticed without any change in the experimental conditions and without specific treatment as introduction to the hemorrhagic disease. Hemorrhagic manifestations and anemia were never observed in rats which had received Peters' eluate (acid-alcohol elution of a yeast extract from charcoal). In several cases, treatment in an early stage with Peters' eluate restored normal conditions.

Examination of the blood of rats in the acute stage of the disease revealed low hemoglobin percentage and low platelet and white blood cell count, with granulopenia. White blood cell counts of less than 1000, with no polymorphonuclear cells, were the rule.

The histological picture of the bone marrow was consistent with the diagnosis of panmyelophthisis, including aplastic anemia and aleukia hemorrhagica.

The state of ascorbic acid in plant tissues. E. J. Reedman (by invitation) and E. W. McHenry, School of Hygiene, University of Toronto.

Previous work in this laboratory has shown that many plant tissues contain vitamin C as free ascorbic acid, dehydroascorbic acid, and in a combined form which is not extracted by customary methods. Chemical estimations of vitamin C do not show the correct values unless all three forms are measured. Combined ascorbic acid is insoluble in trichloracetic and meta phosphoric acids. It can be hydrolyzed by heating or by an approriate acid treatment at room temperature. After hydrolysis the non-ascorbic acid part of the compound is precipitated by trichloracetic acid. This material is protein. After removal of the protein the solution behaves chemically exactly as if it were a solution of pure ascorbic acid. Increased titration values due to measurement of combined ascorbic acid are not attributable to destruction of ascorbic acid oxidase as has been stated by Mack and others. Ascorbic acid, liberated from the protein compound, is oxidized by the oxidase or by chemical means. Spectrophotometric analyses clearly indicate that the reducing substance in the protein compound is ascorbic acid. The characteristic absorption bands are removed by oxidation. Information is not available to indicate the physiological significance of the occurrence of this protein-ascorbic acid compound. Estimation of the amounts of combined ascorbic acid in a number of foods has been made.

The effect of the concentration of dietary egg white on the need for a protective factor given orally or parenterally. Helen T. Parsons, Jane G. Lease (by invitation) and Eunice Kelly (by invitation), Department of Home Economics, University of Wisconsin.

The pellagra-like nutritional disorder in the rat or chick, due to egg white, is characterized by a definite interrelationship between the egg white of the ration and a certain potent factor, i.e., factor X of Boas, not identical with any member of the vitamin B-complex so far clearly characterized.

This interrelationship is such that the greater the concentration of dietary egg white the greater the amount of the factor necessary to cure the nutritional disorder; this is demonstrable when the potent factor is given either orally or parenterally.

The implication of these data is that the interrelationship is metabolic in nature rather than due to changes in the ration mixture or in the digestive tract. Inasmuch as the syndrome has not been reported so far on purified rations containing no egg white, it has not yet been demonstrated that the protective factor is a vitamin or that it is required in normal nutrition.

Substituting egg white autoclaved for 6 hours at 15 pounds pressure for the raw egg white in the physiologically harmful rations leads to prompt cures; that this is due to a removal of the injurious qualities of the egg white rather than to a production of the potent factor by autoclaving is shown by the lack of benefit obtained by feeding autoclaved egg white if the raw egg white remains in the ration together with the autoclaved.

The lactic acid in human blood in relation to the respiratory quotient after the ingestion of hexoses. T. M. Carpenter, E. H. Bensley (by invitation), D. B. Dill and H. T. Edwards (by invitation), Nutrition Laboratory of the Carnegie Institution of Washington, Boston.

The lactic acid content of the venous blood of two human subjects was determined in experiments with 50 to 100 gm. of glucose, fructose, or galactose at 45-minute and 1-hour intervals for 4 hours after ingestion. The respiratory exchange was measured between the taking of the blood samples and the alveolar carbon dioxide followed at 5-minute intervals. Glucose caused a slight increase in the lactic acid for at least 1¼ hours. Galactose caused a fall of the alveolar carbon dioxide for the last 3 of the 4 hours and a very marked increase in lactic acid, but not the same amount with both subjects. The increase in lactic acid was the greatest after the ingestion of fructose and lasted for 2¾ to 3 hours. Corrections of the average respiratory quotients were made from the changes in the alveolar carbon dioxide and the lactic acid. The final results showed that the increases in the respiratory quotients after the ingestion of glucose and galactose were nearly the same but that the increases after fructose were definitely greater than the other two sugars. The end results of the metabolism of glucose and galactose are the same, but the metabolism of fructose is accompanied by either a more rapid combustion of carbohydrate or a greater formation of fat than with the other sugars.

Are endogenous urinary nitrogen and basal metabolism related in the growing rat? U. S. Ashworth (by invitation) and G. R. Cowgill, Laboratory of Physiological Chemistry, Yale University.

In order to test the recently formulated universal law that 2 mg. of endogenous urinary nitrogen are excreted for every calorie of basal heat produced 130 rats of various sizes were used. A measurement was made of the basal metabolism and endogenous nitrogen excretion of each individual.

Approximately linear trends were secured when the logarithms of either basal calories or endogenous nitrogen were plotted against the logarithms of body weight, the correlation coefficients being 0.95 and 0.94, respectively. When basal calories and endogenous nitrogen were correlated with each other the correlation coefficient was found to have a value of 0.87. However, the net correlation coefficient between these two variables was not significantly different from zero when the body weight effect had been eliminated by partial correlation. Indications are then, that the reported proportionality between the basal heat and endogenous nitrogen excretion of growing rats is only apparent and not real, and lies in the relationship of each of these to body weight.

The shape of the curve secured by plotting basal metabolism against endogenous nitrogen for growing rats is very similar to that of the ordinary growth curve. This suggests that during the period of most rapid growth the basal output of heat increases relatively faster than the basal output of nitrogen.

The influence of the percentage of protein upon the thermogenic (specific dynamic) effect and the net energy of the diet. T. S. Hamilton (introduced by H. H. Mitchell) Department of Animal Husbandry, College of Agriculture, University of Illinois.

In a series of twelve paired-feeding experiments, combined with carcass analyses of rats fed, it was found that the growth-promoting values of diets increased as the percentage of protein increased from 4 to about 18; remained constant between 18 and probably about 30%; and then decreased when 42% or more protein was present.

The thermogenic effect, being the total heat calculated to have been produced in excess of the basal, following the ingestion of a unit of food, was found to decrease as the percentage of protein increased from 4 to about 18; remain practically constant for diets containing between 18 and 30% protein; and then increase rapidly when 42% or more protein was present.

The net energy varied inversely with the thermogenic effect, being higher for diets containing between 18 and 30% protein, and lower for diets containing either less than 18 or more than 30% protein.

Percentages of protein varying from 4 to 54 affected neither the muscular activity of the rats nor the basal metabolism, although it was found that the basal metabolic rates of growing rats was readily influenced by the caloric level on which the animals had been subsisting. These data support the conception that the better balanced a diet is, in satisfying an animal's requirements, the smaller will be its thermogenic effect and the greater its net energy value.

The energy metabolism and mechanical efficiency of young boys. Clara M. Taylor (by invitation) and Grace MacLeod, The Nutrition Laboratory of Teachers College, Columbia University.

The energy expenditure of six healthy boys, 9 to 11 years of age, has been measured 1) under standard basal metabolism conditions, 2) while sitting quietly indulging in such activity as would involve only small finger muscles, and 3) while riding a bicycle ergometer of electric brake type.

Basal metabolism was measured by means of various forms of Benedict respiration apparatus. The average deviation of the results from Dreyer predictions was —10.1% (range, —17.7 to + 1.2%); from the Benedict-Talbot standards, + 4.3% (range, — 4.6 to + 14.3%); and from the Harris-Benedict predictions, + 4.6% (range, —2.7 to + 15.4%).

The activity studies were carried out in a respiration chamber patterened after that of Benedict and Ritzman. The Carpenter-Haldane gas analysis apparatus was used with it.

Total work performed in pedaling the bicycle ergometer was determined, as well as carbon dioxide production.

Average energy expenditure in forty-six quiet play periods was 2.47 Cals. per kilogram per hour. This is 53% above the average basal energy expenditure of 1.61 Cals. per kilogram per hour.

Average energy expenditure in thirty-five cycling periods was 5.03 Cals. per kilogram per hour, 212% in excess of the average basal energy expenditure.

Average gross efficiency in the thirty-five cycling periods was 15.8%. If net efficiency is calculated by deducting the basal metabolism it is found to be 23.1%; if calculated by deducting the sitting metabolism it becomes 31.5%.

A new automatic respiration calorimeter, and a study of the influence of sugars on heat production in the rat. W. M. Barrows, Jr. (by invitation), W. R. Murlin (by invitation), Pauline Nutter (by invitation) and John R. Murlin, Department of Vital Economics, University of Rochester.

An automatic respiration calorimeter for small animals has been constructed, which operates upon the following principle:

In addition to the heat produced by the animal, artificial heat is supplied to the interior of the calorimeter by means of an electric heater. The total heat supply to the calorimeter is automatically maintained constant by means of a self-balancing Wheatstone bridge circuit, which is actuated by resistance thermometers in the circulating water and which actuates a motor-driven rheostat. This rheostat is connected in series with the electric heater and with a recording watt meter.

In order to measure the rate of heat production by the animal, it is necessary only to know the (constant) rate of total heat supply, subtract from this the amount supplied electrically, as shown by the watt meter and correct for the latent heat of water vaporized.

The 'indirect heat' may be measured by either the open- or closed-circuit method.

The calorimeter was designed to measure the total heat produced simultaneously by twelve to eighteen rats over long periods.

Results were shown comparing such measurements on paired rats fed two equicaloric diets containing 68% glucose and sucrose, respectively. The heat production during several weeks was identical, expressed on a basis of surface area. Results also were shown comparing the effects of similar diets differing by a small known number of calories.

ABSTRACTS OF PAPERS READ BY TITLE

The chemical composition of the increment of growth at different rates of growth. Marjorie Pickens (by invitation), William E. Anderson and Arthur H. Smith, Laboratory of Physiological Chemistry, Yale University.

This study is an attempt to evaluate progressive changes in body composition during growth under controlled dietary conditions. Four groups of male albino rats of uniform initial age and weight were reared on diets which produced four distinct levels of growth. These included rapid and 'normal' growth, and two levels of retarded growth provided by gliadin-stunted animals and their calorie controls. After the one hundred and tenth day both stunted groups were realimented. The whole bodies of rats at birth, 14 days, and 21 days, and of animals at three different ages from the four dietary groups were analyzed for water, fat, protein, and ash. The average body composition of groups of eight rats of each diet and age showed increase of fat and loss of water with age on all diets, together with approximate constancy of protein content. The greatest variations between individuals of the same group and between different groups were in fat content. Fat, which was present to the extent of 24% in the bodies of the most rapidly growing animals of 230 days of age, represented 40% of the weight gained by these animals between the one hundred and tenth and two hundred and thirtieth days. The proportion of fat in the gains during the period of limited intake of energy was less than half of that in the gains of rapidly growing animals of the same age, after realimentation it increased sharply.

The specificity of galactose as a cataract producing agent. Helen S. Mitchell, Gladys M. Cook (by invitation), Oreana A. Merriam (by invitation), Home Economics Research, Agricultural Experiment Station, Massachusetts State College.

Galactose is now recognized as a unique causative agent in the experimental production of lens opacities in rats. The effect of altering the type and amount of other ingredients in the experimental ration has been investigated. Clinical experience and chemical analyses of lenses have suggested other leads.

The cataract-producing action of lactose or galactose rations was not appreciably altered by 1, type of supplementary carbohydrate (starch, dextrine, sucrose, glucose); 2, type of protein (casein, dried meat, egg albumin, lactalbumin) or adequate amounts (15 and 30%); 3, type of fat (Crisco, butter, mutton tallow, cod liver oil) or amount (2, 11, 22 and 44%); 4, addition of excess cholesterol (2½ and 5%); 5, amount of salt mixture (0, 4 and 10%); 6, excess calcium as lactate (4, 6 and 8%); 7, shifting acid-base balance (4.76% Na citrate, 3.4% NH_4Cl); 8, amount of water ingested; 9, vitamin C administered orally or injected; 10, deficiency or excess of vitamins B_1 and B_2; 11, ingestion of large doses of dinitrophenol; 12, injection of lens antigen (from beef, sheep, guinea pig and rat).

Cataract producing action of galactose was hastened by lowering the protein (5%) and slightly delayed by the addition of cystine (1, 2 and 3%). The protein factor is being further investigated.

Nitrogen metabolism in premature infants: Comparative studies of human and cow's milk. H. H. Gordon (by invitation), M. A. Wheatley (by invitation) and E. Maples (by invitation), Children's Clinic, New York Hospital.

Balance studies of nitrogen and fat were made in six healthy premature male infants, ranging in age from 16 to 34 days and in weight from 1.45 to 2.72 kg. at the onset of observations. Urine and feces were collected separately in eighteen observations of 3 to 9 days (totalling 91 days) following fore periods of constant diet of 3 to 7 days.

Four infants received equivaloric diets of human and cow's milk mixtures containing approximately 125 calories and 2.75 gm. of protein per kilogram of body weight per 24 hours. The average retentions of nitrogen (dietary minus urinary and fecal nitrogen) for these short periods were 0.25 and 0.27 gm./kg./24 hours and the average percentages of utilization ($\frac{\text{nitrogen retained}}{\text{dietary minus fecal nitrogen}}$) 70.9 and 70.4% for human and cows' milk, respectively. These are considerably higher than the figures reported in full term infants under comparable conditions.

Increasing the protein (cows' milk) to 5 and 9 gm./kg./24 hours without increasing the calories increased the nitrogen retained to 0.36 and 0.45 gm./kg./24 hours, respectively, but the percentages of utilization dropped to 51 and 33%.

No defect in absorption of nitrogen was found. The absorption of fat from both human and cows' milk was less than the results reported for full term infants.

Malignant changes in forestomach mucosa of rats related to low protein (casein) diet and prevented with cystine. George R. Sharpless, Department of Laboratories, Henry Ford Hospital.

Hyperplasia of the squamous epithelium of the forestomach was observed in all of ninety-eight hooded and Wistar Institute albino strain rats fed a purified diet containing 4% casein and 5% yeast as sources of protein. This hyperplasia ranged from slight thickening and papilloma formation to extensive keratinization with the formation of epithelial cysts. The basement membrane is frequently broken through and the submucosa and muscle invaded by large hyperchromatic cells many of which are in mitosis.

The type of carbohydrate has no effect on the development of the lesion. Rats having a low food intake did not show as severe type of lesion as those which ate well (7 to 9 gm. daily). Neither vitamin A nor vitamin B deficiency increased the severity of the lesion, and an excess of either vitamin did not prevent it.

With 8% casein a less severe lesion developed after 4 months. Thirty-five rats fed 12% casein showed no lesions in first or second generations. Nineteen rats fed the basal diet with 0.2% cystine had no lesions. Ten milligrams of glutathione per rat daily or 0.2% cysteine hydrochloride did not prevent the lesion.

ROUND TABLE DISCUSSIONS

Total metabolism, insensible perspiration and skin temperature. L. H. Newburgh, presiding.

James D. Hardy opened the discussion on 'skin temperature' by describing his radiometric method of measuring skin temperature with absolute accuracy. The relation of skin temperature to peripheral circulation was discussed by Alan Burton and an index for measurement of this circulation in terms of skin temperature, body temperature and environmental temperature was presented. The application of the method of 'partitional calorimetry' to the measurement of the body heat lost by radiation, convection and evaporation, respectively, was described by A. P. Gagge. J. C. Hinsey concluded the discussion with a summation of the various autonomic nervous processes involved in the thermal responses of skin temperature to the environment.

L. H. Newburgh then introduced the subject of insensible loss of weight. He showed that the adult human being in a comfortable state loses close to 25% of his total heat by vaporization of water. One may therefore calculate total heat elimination from the weight of the water vapor. S. Z. Levine reported his studies on normal and premature infants. Max Kriss studying cattle in the respiration chamber found wide variations in this relationship. James M. Greene showed that this method of determining calorie output of rats, both in the basal and 24 hourly state, is satisfactory under carefully controlled conditions.

Nutrition in the clinic: The ideal liaison between the research worker and the clinician. Frederick F. Tisdall, presiding.

Seventy-four members and guests attended this discussion group. A free discussion ensued which showed the desirability of the clinicians being familiar with the rapid increase of scientific information relative to nutritional problems being obtained by the laboratory workers. On the other hand, the discussion served to give the laboratory workers a little insight into the difficulties which beset the clinicians. The closest possible cooperation between these two groups of workers was shown to be most desirable.

The discussion indicated the possible need for more of some of factors in the vitamin B-complex in the average daily diet; that there is great need for further clinical and laboratory work to be done on vitamin C in order to determine the daily human needs of this food element; recent laboratory work on vitamin D was reviewed and the fact that the human reacts to the different forms of vitamin D essentially in the same manner as the rat was emphasized, and the importance of the available iron and not the total iron in human nutrition was stressed.

At the conclusion of the meeting a vote was taken to find out whether the members present were in favor of discussion groups rather than a set address or series of papers. The vote was unanimously in favor of the discussion groups.

Methods of judging nutritional status. Lydia J. Roberts, presiding.

Lydia J. Roberts introduced the round table on the estimation of the nutritive status of children by reviewing the tests now in use and their deficiencies as well as by indicating the difficulties in devising objective physiological, chemical or

anthropometric estimations. Genevieve Stearns discussed the value of the biophotometer for measuring the adequacy of vitamin A intake and utilization and factors affecting its accuracy. Three thousand units of vitamin A had been found to be sufficient for boys 11 to 12 years. C. E. Palmer has not found the biophotometer exact in selecting children on repeated tests, but Genevieve Stearns explained that the method selected subjects with wider margins of adjustment rather than very fine.

Helen Hunscher described some of the possible benefits which might be derived from the use of estimations of skeletal maturity and degree of bone mineralization as judged by serial roentgen studies as means of selecting children for use in metabolic balance studies. Amy Daniels and Julia P. Outhouse contributed to this subject.

Gilbert Dalldorf opened the discussion on physiological means for measuring the adequacy of intake and utilization of vitamin C by human subjects. Estelle Hawley described the method for determining the daily requirement needed beyond that for saturation of body tissues by excretion studies. Fifty milligrams daily intake of ascorbic acid is suggested as a standard. R. A. Sloan reported on interrelated studies on rate of excretion, storage and level of vitamin C in the blood along with x-ray and capillary resistance tests. He indicated many factors which must be observed in these tests to secure reliable estimations. V. E. Levine described his experiences in measuring the vitamin deficiencies of the Eskimos.

It was concluded in this group discussion that there was a great need for adequate objective methods of evaluation of physical status as related to human nutrition.

Interconversion of foodstuffs. John R. Murlin, presiding.

Various aspects of the controversy over fat to carbohydrate occupied the larger part of the interconversion discussion under the leadership of J. R. Murlin. It was opened by H. J. Deuel, Jr. with an account of the transformation of amino acids, glycerol and odd-carbon fatty acids into carbohydrate as judged by liver glycogen analyses instead of the phlorhizin method. Data concerning a general correlation of the glycogen content of the body and the ability to oxidize carbohydrate were presented by W. H. Chambers. S. Soskin then spoke on the implications of considering the D: N ratio as derived from both fat and protein and the dominating position of the liver. The agreement between the chemical analyses of pupating insects and their low respiratory quotients at this time was shown by F. A. Hitchcock to support the fat to carbohydrate hypophysis. H. E. Himwick referred to the influence of methylene blue in lowering the R.Q. by the mechanism of the oxidation of hydrogen and then he broke up the quotient of the whole body into fractions contributed by the parts according to tissue determinations. This discussion led into the work of M. Elizabeth Marsh and the unsuccessful attempts to explain quotients of 0.58 in liver slices by changes in acetone bodies, glycerol or protein metabolism. T. M. Carpenter pointed out the constancy of the height of the R.Q. after small or large doses of fructose, unaffected by muscular exercise, and emphasized the possible acid-base relationships. The discussion was closed on a less controversial note with a review by L. S. Palmer of the evidence for the conversion of carbohydrate to fat. About 150 people were in attendance, rather too many for intimate give and take in a group discussion.

Comparative nutrition—species differences as regards nutritive requirements and the effects of harmful food substances. L. A. Maynard, presiding.

After a few introductory remarks by L. A. Maynard, the following papers were presented:

1. Small animal studies as guides in human nutrition, by Arthur H. Smith, Department of Physiological Chemistry, Yale University.

2. Experiments with chicks in their relation to other species, by L. C. Norris, Department of Poultry Husbandry, Cornell University.

3. The nutritive requirements of the lower forms, by R. M. Melampy, Southern States Bee Culture Field Laboratory, Baton Rouge.

4. The vitamin requirements of guinea pigs and rabbits, by A. G. Hogan, Department of Agricultural Chemistry, University of Missouri.

Each paper was followed by rather vigorous discussion in which many of the sixty members present took part.

Expression of opinion from individuals indicated definite approval of this type of meeting.

Vitamin nomenclature. E. M. Nelson, presiding.

The meeting was called to order by the chairman whose opening remarks dealt with the scope of the problem. It was pointed out that there are more substances having the property of a vitamin than there are letters in the alphabet, and that there is obviously no hope for a system of nomenclature which will be so simple that the character of all the compounds can be readily comprehended. This was followed by the prearranged program in which W. C. Rose, Franklin C. Bing and A. D. Emmett partook. W. C. Rose stressed the inalienable right to a discoverer in naming a compound and also stated that anyone possessing such a right should give due recognition to systems of nomenclature and to problems which may arise in the use of names having wide application. F. C. Bing discussed the objections of the Council on Pharmacy and Chemistry to names which are therapeutically suggestive. A. D. Emmett emphasized the problems of the pharmaceutical manufacturer in teaching the physican and the layman the significance of names chosen for various compounds. Changes of names led to rather difficult situations.

In the discussion which followed particular attention was given to the desirability of giving specific names to vitamin A and to the various forms of vitamin D. There was no disposition on the part of the group to make definite recommendations with respect to these vitamins but the discussion will be very helpful to the committee on nomenclature. There was considerable discussion of the recommendations for vitamin B nomenclature which were approved at the business meeting of the institute. These recommendations met with general approval.

In discussing the objection of the Council on Pharmacy and Chemistry to names which are therapeutically suggestive, the chairman stated that he was cognizant of the fact that the council had been criticized in the past for its selection of names. He emphasized, however, that recognition must be given to the fact that there is a sound basis for objecting to names which are therapeutically suggestive. The council must, of course, interpret its own rules, and there is no need

for confusion if the position of the council is clearly understood and there is satisfactory cooperation between all interested groups.

The meeting was attended by approximately forty people of whom nearly one-half took part in the discussion. No one left the meeting before formal adjournment except those who had previously obtained permission to do so.

Data assembled by George R. Cowgill on the vitamin requirements of man. General meeting evening of April 21, 1937, Mary Swartz Rose, presiding.

Average American diet

	Vit./Cal.
Man (154 pounds) needs	1.97
Sherman's estimated averaged diet yields	2.74
121 family diets calculated separately averaged	2.77

Steffanson and Anderson ate an 'exclusive meat diet' for a period of 1 year and did not develop beriberi.

	Vit./Cal.
The diet yielded	2.77
Steffanson needed	1.96
Anderson needed	1.62

Sufficient vitamin B was indeed present.

Vitamin B and growth. Growth of rat requires from three to five times more than does maintenance (Dann). Children require for optimal retention of vitamin B from six to seven times the beriberi preventing minimum (Knott). Children in Toronto were not getting this amount (Ross and Summerfeldt).

Vitamin B for pregnancy and lactation. League of Nations Commission, 1935, recommends 150 to 250 International units per day, which is about 5 to 6 units per 100 cal. per day. This seems low. Safer minimum: at least 10 units per 100 cal. per day.

Vitamin B for the infant. Intake based on amount from human milk with maximum B content would be about 80 International units per day.

American Public Health Year Book, 1934–1935, recommends minimum of 50 units daily.

Vitamin A for pregnancy and lactation. League of Nations Commission, 1935, recommends: 9000 International units per day which is about 250 to 300 units per 100 cal. per day.

Vitamin A for the infant. Two thousand International units per day (Am. Public Health Year Book, 1934–1935). This amount means about 400 units per 100 cal. per day.

Good human milk contains 280 to 710 units per 100 cal. Cow's milk maximum is about 90 units per 100 cal.

Vitamin C in milk. Human milk maximum is about 1.4 to 1.6 International units per cubic centimeter. Thus the very young baby could get 1000 to 1200 units per day or 200 to 240 units per 100 cal. per day, or 2.75 units per kilogram per day. This is much more than most infants receive—cow's milk has much less, only 0.4 to 0.5 units per cubic centimeter.

Vitamin C for pregnancy and lactation. League of Nations Commission, 1935, recommends: 900 to 1100 International units per day or about 30 units per 100 cal. per day.

Vitamin D for the infant. To prevent rickets: 700 International units per day (Am. Public Health Year Book, 1934–1935) or 140 units per 100 cal. per day or 200 units per kilogram per day.

PAST MEETINGS, OFFICERS AND DECEASED MEMBERS

Organization meeting—1933—Henry C. Sherman, Chairman

1934 New York city
President: L. B. Mendel
Vice-President: H. C. Sherman
Secretary-Treasurer: J. R. Murlin
Council: E. F. DuBois, M. S. Rose

1935 Detroit
President: J. R. Murlin
Vice-President: E. F. DuBois
Secretary: I. G. Macy
Treasurer: W. M. Boothby
Council: A. F. Morgan, A. H. Smith, R. M. Bethke

1936 Washington
President: J. R. Murlin
Vice-President: E. F. DuBois
Secretary: I. G. Macy
Treasurer: G. R. Cowgill
Council: R. M. Bethke, L. A. Maynard, A. H. Smith

1937 Memphis
President: E. F. DuBois
Vice-President: M. S. Rose
Secretary: I. G. Macy
Treasurer: G. R. Cowgill
Council: R. M. Bethke, L. A. Maynard, C. A. Elvehjem

Deceased members of American Institute of Nutrition

Graham Lusk, July 18, 1932
Alfred F. Hess, December 5, 1933
A. W. Rowe, December 6, 1934
Lafayette B. Mendel, December 9, 1935
Winfred W. Braman, March 24, 1937
W. McKim Marriott, November 11, 1936

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